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on

METHODS FOR PREDICTING FUNCTIONAL AND STRUCTURAL
PROPERTIES OF POLYPEPTIDES USING SEQUENCE MODELS

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METHODS FOR PREDICTING FUNCTIONAL AND STRUCTURAL
PROPERTIES OF POLYPEPTIDES USING SEQUENCE MODELS

This application claims benefit of provisional application serial no. 60/_____, filed

5 December 29, 2000, which was converted from U.S. Serial No. 09/753,020, filed December 29, 2000, and which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates generally to
10 interactions between ligands and polypeptides and more specifically to determining structure-related properties of a ligand when bound to different polypeptides.

Structure determination plays a central role in chemistry and biology due to the correlation between the
15 structure of a molecule and its function. Although a full understanding of this correlation is not yet established, one can gain insight into the function of a molecule from its deduced structure. Thus, the structure can provide a strong basis for formulating experiments to
20 determine function. Conversely, the eventual disclosure of a structure for a well studied molecule can have a significant effect in converging apparently disparate observations of function into a consistent description of the molecule's activity.

25 Practical applications which are becoming increasingly dependent upon structure information include, for example, the production of therapeutic drugs. Therapeutic drugs can be designed by synthesizing

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a molecule that mimics a ligand known to interact with a target receptor. Alternatively, a therapeutic drug can be designed by computer assisted methods in which a molecule is designed to dock to a binding site on a
5 receptor of known structure. By structure-based methods such as these, lead compounds can be identified for further development.

Using a similar structure based approach a receptor can be engineered to yield improved or novel
10 functions. For example, changes can be made at a ligand binding site in a polypeptide receptor based on the known structure of the receptor. Given that a polypeptide receptor can contain hundreds or even thousands of amino acid residues, of which only a few may contact a ligand,
15 structural information is useful in identifying where changes should be made in the polypeptide to alter ligand binding. Polypeptide receptors engineered as such can be used for a variety of practical applications including, for example, industrial catalysis, therapeutics, and
20 bioremediation.

Although methods for structure determination are evolving, it is currently difficult, costly and time consuming to determine the structure of a polypeptide or ligand. It can often be even more difficult to produce a
25 polypeptide-ligand complex in a condition allowing determination of a structure for the bound complex. Resorting to determining a structure for the receptor individually can have limited value, particularly if the location of ligand binding is difficult to identify due
30 to the large size of most polypeptide receptors. Similarly, determination of a structure of an unbound

ligand can have limited usefulness because an unbound ligand has multiple conformations and the most stable conformation of an unbound ligand is often different from its conformation when bound to a receptor.

5 Theoretical modeling of ligand-polypeptide interactions is one alternative that has been attempted in cases where the structure of the polypeptide-ligand complex is not available. In this approach a ligand is fitted to a structure of a polypeptide. The polypeptide
10 structure used can be determined empirically or theoretically. Theoretical determination of a hypothetical molecular structure for a polypeptide by *ab initio* methods is a relatively undeveloped method.
Another theoretical approach, referred to as homology
15 modeling, has been used to infer structure based on comparison with molecules of known structure.

The successful application of homology modeling to determining polypeptide-ligand interactions relies upon choosing a correct polypeptide template for
20 comparison. In most cases criteria for comparison are unavailable or unreliable. For example, it is common to produce a hypothetical structure of a target polypeptide based on the empirically determined structure of a template polypeptide having similar sequence. However,
25 similarities in sequence do not always yield similar structures and conversely, similar structures have been observed for two polypeptides having significantly diverged sequences.

Thus, there exists a need for efficient methods
30 to identify properties of a ligand that confer binding

specificity for polypeptide receptors. A need also exists for methods to classify polypeptides and ligands according to structural characteristics. The present invention satisfies this need and provides related
5 advantages as well.

SUMMARY OF THE INVENTION

The invention provides a method for identifying a polypeptide that binds a ligand. The method includes the steps of (a) comparing a sequence of a polypeptide to
10 a sequence model for polypeptides that bind a ligand, wherein the sequence model comprises representations of amino acids consisting of a subset of amino acids, the subset of amino acids having one or more atom within a selected distance from a bound ligand in the polypeptides
15 that bind the ligand; and (b) determining a relationship between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence model identifies the polypeptide as a polypeptide that binds the ligand.

20 The invention also provides a method for identifying a member of a pharmacofamily. The method includes the steps of (a) comparing a sequence of a polypeptide to a sequence model for polypeptides of a pharmacofamily; and (b) determining a relationship
25 between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence model identifies the polypeptide as a member of the pharmacofamily.

The invention also provides a method for identifying a member of a pharmacofamily, wherein the method includes the steps of (a) comparing a sequence of a polypeptide to a sequence model and a differential sequence model; and (b) determining a relationship between the sequence and the sequence models, wherein a correspondence between the sequence and the sequence models identifies the polypeptide as a member of the pharmacofamily.

10

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows pharmacoclusters identified from a database of 156 bound structures of nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate. Structures were generated using the overlay function in INSIGHT98 (Molecular Simulations Inc., San Diego, CA).

Figure 2 shows the nomenclature used herein for atom names in the NAD(P) molecule.

Figure 3 shows conformer models with interacting atoms from bound polypeptide and ordered waters overlayed. Models in parts A through H were derived from pharmacoclusters 1-8, respectively as described in the Examples. Overlayed atoms and waters are identified as either hydrogen bond donors (donors), hydrogen bond acceptors (acceptors), sulfurs (sulfurs), waters (waters), or atoms that can be hydrogen bond acceptors or hydrogen bond donors (acceptors/donors) according to the legend in part A.

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Figure 4 shows a portion of a 2D [$^1\text{H}, ^1\text{H}$] NOESY spectrum recorded with a 0.2 ml sample of 1 mM NADP and 200 μM of enzyme 1-deoxy D-xylulose 5-phosphate reductoisomerase (DOXP). Atoms are identified according to Figure 2. Spectra are reported as parts per million (ppm). Since ligand is in fast exchange and in excess over polypeptide, cross peaks represent transferred NOEs.

Figure 5 shows high affinity binding of compound TTE0001.001.A07 to polypeptide enzymes of pharmacofamily 1 (panel A) and pharmacofamily 8 (panel B). Double reciprocal plots of reaction rate versus concentration of NADH (panel A) or NADPH (panel B) are shown for each enzyme in the presence of various concentrations of compound TTE0001.001.A07. Concentrations of compound TTE0001.001.A07 shown to the right of the plot A correspond 7.1 μM (open triangles), 3.6 μM (closed triangles), 1.8 μM (open circles) and no added compound (closed circles). Concentrations of compound TTE0001.001.A07 shown to the right of the plot B correspond 56.2 μM (open triangles), 37.5 μM (closed triangles), 18.7 μM (open circles) and no added compound (closed circles). Inhibitory dissociation constants (K_{1s}) determined from the data are shown in the upper left corner of the respective plot.

Figure 6 shows high affinity binding of compound TTE0001.002.D02 to a polypeptide enzyme of pharmacofamily 1. A double reciprocal plot of reaction rate versus concentration of NADH is shown for the enzyme in the presence of various concentrations of compound TTE0001.002.D02. Concentrations of compound

TTE0001.002.D02 shown to the right of the plot A correspond 20.6 μM (open triangles), 13.7 μM (closed triangles), 6.9 μM (open circles) and no added compound (closed circles). An inhibitory dissociation constant 5 (K_{1s}) determined from the data is shown in the upper left corner of the plot.

Figure 7 shows a pharmacophore model derived from the coordinates presented in Table 3 for pharmacofamily 1. Figure 7A shows a feature of the 10 pharmacophore model including a volume defining the shape of conformer model 1 which is indicated by grey spheres and superimposed on the conformer model having coordinates listed in Table 3C. Figure 7B shows three features of the pharmacophore model including a 15 hydrophobic region of the nicotinamide ring, a hydrogen bond acceptor positioned at the averaged coordinates for the location of 17 hydrogen bond acceptors in the polypeptides of pharmacofamily 1, and a hydrogen bond donor positioned where a hydrogen bond donor of a ligand 20 would be expected to have favorable interactions with hydrogen bond acceptors observed in 11 out of 17 of the polypeptides in pharmacofamily 1. Figure 7C shows a combination of features of figures 7A and 7B present in a 25 pharmacophore model and superimposed on the conformer model.

Figure 8 shows a plot of $-\ln(E)$ vs. L for the results of searching the PDB with a Hidden Markov Model trained with sequences from pharmacofamily 5. E is the Expectation value and L is the location of identified 30 sequences in a list ranked by E value. Identified sequences and respective E values are listed in Table 12.

True positives are plotted as diamonds and false positives are plotted as circles.

Figure 9 shows a plot of $-\ln(E)$ vs. L for the results of searching the PDB with a Hidden Markov Model
5 trained with a first set of sequences from pharmacofamily 3. E is the Expectation value and L is the location of identified sequences in a list ranked by E value.
Identified sequences and respective E values are listed
in Table 13. True positives are plotted as diamonds and
10 false positives are plotted as circles.

Figure 10 shows a plot of $-\ln(E)$ vs. L for the results of searching the PDB with a Hidden Markov Model trained with a second set of sequences from pharmacofamily 3. E is the Expectation value and L is
15 the location of identified sequences in a list ranked by E value. True positives are plotted as diamonds and false positives are plotted as circles.

Figure 11 shows a sequence alignment made from a structural overlay of pharmacofamily 1. Amino acids
20 shown correspond to those which are within regions that overlap in the structural overlay. All bolded letters are within 4.5 Angstroms from a ligand binding site. Underlining indicates proximity to a cofactor ligand and/or substrate ligand as follows: bold underling
25 indicates proximity to a bound cofactor, double underling indicates proximity to a bound substrate, and dotted underling indicates proximity to both bound cofactor and bound substrate.

Figure 12 shows a plot of $-\ln(E)$ vs. L for the results of searching the PDB with a Hidden Markov Model trained with sequences from pharmacofamily 1. E is the Expectation value and L is the location of identified sequences in a list ranked by E value. Identified sequences and respective E values are listed in Table 15. True positives are plotted as diamonds and false positives are plotted as circles.

Figure 13 shows a plot of $-\ln(E)$ vs. L for the results of a differential search of the PDB with a first Hidden Markov Model trained with sequences from pharmacofamily 1 and a second Hidden Markov Model trained with sequences including residues proximal to a bound ligand in polypeptides of pharmacofamily 1. E is the Expectation value and L is the location of identified sequences in a list ranked by E value. Identified sequences and respective E values are listed in Table 16. True positives are plotted as diamonds and false positives are plotted as circles.

Figure 14 shows the data of Figure 12 overlayed with XCorr values calculated for each sequence. XCorr values are plotted as triangles, true positives are plotted as squares and false positives are plotted as circles.

25

DETAILED DESCRIPTION OF THE INVENTION

The invention provides pharmacoclusters and methods for identifying a pharmacocluster from bound conformations of a ligand bound to different polypeptides. The methods are applicable for identifying

a conformation-dependent property of a ligand based on bound conformations of the ligand in a pharmacocluster. The methods are also applicable for classifying polypeptides, from a family of polypeptides that bind the 5 same ligand, into pharmacofamilies based on bound conformations of the ligand. Accordingly, methods are provided for grouping polypeptides into pharmacofamilies by determining bound conformations of a ligand or a conformation-dependent property of a ligand independent 10 of a determination of the structure of the polypeptide. An advantage of classifying polypeptides according to bound conformations of a ligand is that a pharmacofamily is likely to contain polypeptides having greater binding specificity for a particular molecule than other 15 polypeptides in the same family. Thus, the methods allow identification of a pharmacofamily that can specifically interact with a particular therapeutic agent or drug.

Additionally, the methods of the invention can be used to determine a conformer model or pharmacophore 20 model based on a bound conformation or conformation-dependent property of a ligand bound to polypeptides in a pharmacofamily. The invention is therefore advantageous in providing a model for the design and identification of therapeutic compounds having specificity for a 25 pharmacofamily of polypeptides.

Further, the methods of the invention can be used to identify structural properties and ligand binding 30 properties of polypeptides based on comparison of their sequences to polypeptides in one or more pharmacofamilies. An advantage of the invention is that ligand binding properties can be identified for

polypeptides in a database for which sequence information is readily available but structural and/or functional properties are incompletely known or unavailable.

Another advantage of the invention is that the
5 methods provide a correlation between ligand conformation, a parameter that is relatively easy to measure, and polypeptide structure, a parameter of tremendous value but often difficult to measure. Therefore, the methods of the invention can be used to
10 determine structural characteristics of a polypeptide based on a conformation-dependent property of a bound ligand.

As used herein, the term "pharmacocluster" refers to a collection of substantially the same bound
15 conformations of a ligand, or portion thereof, bound to two or more polypeptides. A member conformation of a pharmacocluster can have (1) a conformation that is more similar to an average conformation of the members in its pharmacocluster than to any other pharmacocluster and (2)
20 a conformation that is more similar to an average conformation of the members in its own pharmacocluster than the most similar average structures from different pharmacoclusters are to each other, wherein the pharmacoclusters consist of conformations of the same
25 ligand or portion thereof. The pharmacocluster is determined for a ligand bound to different polypeptides but does not require that a structure of the polypeptide be known or included as part of a bound conformation of a ligand. A bound conformation of a ligand can include the
30 entire ligand structure or selected atoms including a portion of the complete atomic composition of the ligand

so long as the number of atoms provides sufficient information to distinguish one pharmacocluster from another. A pharmacocluster can include both the bound conformations of a ligand, or portion thereof, and one or 5 more atoms that both interact with the ligand and are from a bound polypeptide. Thus, a pharmacocluster can include conformational information of 1 or more, 2 or more, 5 or more, 10 or more, 20 or more, 30 or more, 40 or more, 50 or more or 100 or more atoms of a ligand 10 bound conformation.

Accordingly, portions of bound conformations of two or more different ligands can be included in a ligand pharmacocluster so long as the portions selected from each ligand have a core bound conformation that is 15 substantially the same. A core bound conformation can consist of portions of bound conformations of ligands wherein the portions have identical structural formula and conformation. A core bound conformation can also consist of portions of bound conformations of ligands 20 wherein the portions have different structural formulas so long as the portions have substantially the same conformation. The structural formula, as it is understood in the art, is a 2 dimensional representation of a molecule that identifies the atoms and covalent 25 bonds between each atom in the molecule. The structural formula does not necessarily include information sufficient to determine conformation of a molecule. For example, a common structural formula representation of cyclohexane can be a hexagon with 2 hydrogens attached to 30 each carbon being in equivalent positions. However, a stable conformation of cyclohexane in solution may appear as a "chair" or "boat" shape with hydrogens in either

axial or equitorial positions relative to the molecular plane.

As used herein, the term "conformation-dependent property," when used in reference to a ligand, 5 refers to a characteristic of a ligand that specifically correlates with the three dimensional structure of a ligand or the orientation in space of selected atoms and bonds of the ligand. Thus, a ligand bound to a polypeptide in a distinct conformation will have at least 10 one unique conformation-dependent property correlated with the bound conformation of the ligand. A conformation-dependent property can be derived from or include the entire ligand structure or selected atoms and bonds, including a fragment or portion of the complete 15 atomic composition of the ligand. A conformation-dependent property that includes selected atoms and bonds of a ligand can include 2 or more, 3 or more, 5 or more, 10 or more, 15 or more, 20 or more, 25 or more, or 50 or more atoms of a bound conformation of a ligand.

20 A characteristic that specifically correlates with a three dimensional structure of a ligand is a characteristic that is substantially different between at least two different bound conformations of the same ligand and, therefore, distinguishes the two different 25 bound conformations. A conformation-dependent property can include a physical or chemical characteristic of a ligand, for example, absorption and emission of heat, absorption and emission of electromagnetic radiation, rotation of polarized light, magnetic moment, spin state 30 of electrons, or polarity. A conformation-dependent property can also include a structural characteristic of

a ligand based, for example, on an X-ray diffraction pattern or a nuclear magnetic resonance (NMR) spectrum. A conformation-dependent property can additionally include a characteristic based on a structural model, for example, an electron density map, atomic coordinates, or x-ray structure. A conformation-dependent property can include a characteristic spectroscopic signal based on, for example, Raman, circular dichroism (CD), optical rotation, electron paramagnetic resonance (EPR), infrared (IR), ultraviolet/visible absorbance (UV/Vis), fluorescence, or luminescence spectroscopies. A conformation-dependent property can also include a characteristic NMR signal, for example, chemical shift, J coupling, dipolar coupling, cross-correlation, nuclear spin relaxation, transferred nuclear Overhauser effect, or combinations thereof. A conformation-dependent property can additionally include a thermodynamic or kinetic characteristic based on, for example, calorimetric measurement or binding affinity measurement.

Furthermore, a conformation-dependent property can include characteristic based on electrical measurement, for example, voltammetry or conductance.

As used herein, "selected" conformation-dependent properties are identified to form a set of conformation-dependent properties that can include, for example, the entire set of conformation-dependent properties associated with the bound conformations of a ligand in a pharmacocluster or a subset of conformation-dependent properties associated with the bound conformations of a ligand in a pharmacocluster, so long as the subset of conformation-dependent properties are sufficient to identify a unique conformation of the

ligand. A selected conformation-dependent property can include any of the above described properties, for example, a physical or chemical property, structural data, a structural model, a spectroscopic signal, a 5 thermodynamic or kinetic measurement or an electrical measurement.

As used herein, the term "bound conformation," when used in reference to a ligand, refers to the location of atoms of a ligand relative to each other in 10 three dimensional space, where the ligand is bound to a polypeptide. The location of atoms in a ligand can be described, for example, according to bond angles, bond distances, relative locations of electron density, probable occupancy of atoms at points in space relative 15 to each other, probable occupancy of electrons at points in space relative to each other or combinations thereof.

As used herein, a "selected" bound conformation refers to a set of bound conformations that can include, for example, the entire set of defined bound 20 conformations or a subset of bound conformations of a ligand.

As used herein, the term "clustering" refers to assigning related bound conformations of a ligand, or portion thereof, into a first collection such that the 25 conformations residing in the first collection can be overlaid with substantial overlap and bound conformations from two different collections cannot be overlaid with a better overlap than that resulting from members of the first collection. Exemplary clustering of ligand 30 conformations are disclosed herein (see Example I).

- As used herein, the term "ligand" refers to a molecule that can specifically bind to a polypeptide. Specific binding, as it is used herein, refers to binding that is detectable over non-specific interactions by quantifiable assays well known in the art. A ligand can be essentially any type of natural or synthetic molecule including, for example, a polypeptide, nucleic acid, carbohydrate, lipid, amino acid, nucleotide or any organic derived compound. The term also encompasses a cofactor or a substrate of a polypeptide having enzymatic activity, or substrate that is inert to catalytic conversion by the bound polypeptide. Specific binding to a polypeptide can be due to covalent or non covalent interactions.
- As used herein, the term "bound to two or more polypeptides," when used in reference to a ligand is intended to refer to two or more complexes consisting of a ligand and a polypeptide. A complex can include, for example, a single ligand bound to a single polypeptide. A complex can also include a single ligand bound to more than one polypeptides including, for example, a complex in which a ligand is bound at the interface of interacting polypeptides. A complex can also include multiple ligands, however, conformation dependent properties of all ligands of the complex need not be identified. A complex results from a specific interaction between a polypeptide and a ligand.

As used herein, the term "substantially the same," when used in reference to bound conformations of a ligand, or portion thereof, is intended to refer to two or more bound conformations that can be overlaid upon

each other in 3 dimensional space such that all corresponding atoms between the two conformations are overlapped. Accordingly, "substantially different" bound conformations cannot be overlaid upon each other in 3-dimensional space such that all corresponding atoms between the two bound conformations are overlapped.

As used herein, the term "polypeptide" is intended to refer to a peptide polymer of two or more amino acids. The term is similarly intended to include polymers containing amino acid stereoisomers, analogues and functional mimetics thereof. For example, derivatives can include chemical modifications of amino acids such as alkylation, acylation, carbamylation, iodination, or any modification which derivatizes the polypeptide. Analogues can include modified amino acids, for example, hydroxyproline or carboxyglutamate, and can include amino acids, or analogs thereof, that are not linked by peptide bonds. Mimetics encompass chemicals containing chemical moieties that mimic the function of the polypeptide regardless of the predicted three-dimensional structure of the compound. For example, if a polypeptide contains two charged chemical moieties in a functional domain, a mimetic places two charged chemical moieties in a spatial orientation and constrained structure so that the corresponding charge is maintained in three-dimensional space. Thus, all of these modifications are included within the term "polypeptide" so long as the polypeptide retains its binding function.

As used herein, the term "root mean square deviation," or RMSD, refers to a standard deviation which quantifies the structural variability in a population of

bound conformations of a ligand. The term is intended to be consistent with its meaning as understood in the art as described for example in Doucet and Weber, Computer-Aided Molecular Design: Theory and Applications, Academic Press, San Diego CA (1996).

As used herein, the term "family," when used in reference to characterizing polypeptides having ligand binding activity, is intended to refer to polypeptides that can bind to the same ligand, or portion thereof. A 10 polypeptide family can contain polypeptides having binding activity for a common ligand with sufficient affinity, avidity or specificity to allow measurement of the binding event. As defined herein a "member" of a 15 polypeptide family refers to an individual polypeptide that can be classified in a polypeptide family because the polypeptide binds a ligand, or portion thereof, that binds another polypeptide in a polypeptide family. The bound conformations of a ligand bound by individual members of a family can be substantially the same or 20 different from each other.

As used herein, the term "pharmacofamily," when used in reference to polypeptides, is intended to refer to polypeptides that can be classified together in a population because they individually bind a ligand such 25 that the ligand is bound in substantially the same conformation. As defined herein a "member" of a polypeptide pharmacofamily refers to an individual polypeptide that is classified in a polypeptide pharmacofamily because the polypeptide binds a 30 conformation of a ligand that is substantially the same

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as a conformation of the ligand bound to another polypeptide in the pharmacofamily.

As used herein, the term "grouping" refers to assigning related polypeptides into a family or 5 pharmacofamily such that the polypeptide members of a family bind the same ligand and the polypeptide members of a pharmacofamily bind substantially the same bound conformation of a ligand.

As used herein, the term "fold," when used in 10 reference to a polypeptide, refers to a specific geometric arrangement and connectivity of a combination of secondary structure elements in a polypeptide structure. Secondary structure elements of a polypeptide that can be arranged into a fold including, for example, 15 alpha helices, beta sheets, turns and loops are well known in the art. Folds of a polypeptide can be recognized by one skilled in the art and are described in, for example, Branden and Tooze, Introduction to protein structure, Garland Publishing, New York (1991) 20 and Richardson, Adv. Prot. Chem. 34:167-339 (1981).

As used herein, "modeling the three dimensional structure" when used in reference to a polypeptide refers to determining a conformation for a polypeptide. A conformation of a polypeptide can be determined, for 25 example, from empirical data specifying structure or from a compared conformation used as a template. A conformation can be determined at any desired level of resolution sufficient to identify, for example, overall shape of a polypeptide, tertiary structure elements, 30 secondary structure elements, polypeptide backbone

structure, amino acid residue identity or location of individual atoms.

As used herein, the term "structural model," when used in reference to a polypeptide, refers to a 5 representation of a 3 dimensional structure of a polypeptide. A structural model can be determined from empirical data derived from, for example, X-ray crystallography or nuclear magnetic resonance spectroscopy. A structural model can also be derived 10 from a theoretical calculation including, for example, comparison to a known structure or *ab initio* molecular modeling. A representation of a structural model can include, for example, an electron density map, atomic coordinates, x-ray structure model, ball and stick model, 15 density map, space filling model, surface map, Connolly surface, Van der Waals surface or CPK model.

As used herein, the term "conformer model" refers to a representation of points in a defined coordinate system wherein a point corresponds to a 20 position of an atom in a bound conformation of a ligand. The coordinate system is preferably in 3 dimensions, however, manipulation or computation of a model can be performed in 2 dimensions or even 4 or more dimensions in cases where such methods are preferred. A point in the 25 representation of points can, for example, correlate with the center of an atom. Additionally, a point in the representation of points can be incorporated into a line, plane or sphere to include a shape of one or more atom or volume occupied by one or more atom. A conformer model 30 can be derived from 2 or more bound conformations of a ligand. For example a conformer model can be generated

from 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 10 or more, 15 or more, 20 or more or 25 or more bound conformations of a ligand.

- As used herein, the term "average structure,"
- 5 when used in reference to bound conformations of a ligand in a pharmacocluster, refers to conformer model, derived by superimposing the bound conformations of a ligand in a pharmacocluster, and determining an average location in space for corresponding atoms.
- 10 As used herein, the term "pharmacophore model" refers to a representation of points in a defined coordinate system wherein a point corresponds to a position or other characteristic of an atom or chemical moiety in a bound conformation of a ligand and/or an
- 15 interacting polypeptide or ordered water. An ordered water is an observable water in a model derived from structural determination of a polypeptide. A pharmacophore model can include, for example, atoms of a bound conformation of a ligand, or portion thereof. A
- 20 pharmacophore model can include both the bound conformations of a ligand, or portion thereof, and one or more atoms that both interact with the ligand and are from a bound polypeptide. Thus, in addition to geometric characteristics of a bound conformation of a ligand, a
- 25 pharmacophore model can indicate other characteristics including, for example, charge or hydrophobicity of an atom or chemical moiety. A pharmacophore model can incorporate internal interactions within the bound conformation of a ligand or interactions between a bound
- 30 conformation of a ligand and a polypeptide or other receptor including, for example, van der Waals

interactions, hydrogen bonds, ionic bonds, and hydrophobic interactions. A pharmacophore model can be derived from 2 or more bound conformations of a ligand. For example a conformer model can be generated from 3 or 5 more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 10 or more, 15 or more, 20 or more or 25 or more bound conformations of a ligand.

- A point in a pharmacophore model can, for example, correlate with the center of an atom or moiety.
- 10 Additionally, a point in the representation of points can be incorporated into a line, plane or sphere to indicate a characteristic other than a center of an atom or moiety including, for example, shape of an atom or moiety or volume occupied by an atom or moiety. The coordinate system of a pharmacophore model is preferably in 3 dimensions, however, manipulation or computation of a model can be performed in 2 dimensions or even 4 or more dimensions in cases where such methods are preferred.
- 15 Multidimensional coordinate systems in which a pharmacophore model can be represented include, for example, cartesian coordinate systems, fractional coordinate systems, or reciprocal space. The term pharmacophore model is intended to encompass a conformer model.

25 As used herein, the term "moiety" refers to a group of atoms that form a part or portion of a larger molecule. A moiety can consist of any number of atoms in a portion of a ligand and can correlate with a physical or chemical property conferred upon the ligand by the 30 combined atoms. Exemplary moieties of a nicotinamide adenine dinucleotide ligand include a phosphate,

nicotinamide ring, amino group, amide group or ribose ring. In addition, a nicotinamide adenine dinucleotide group can be a moiety. For example, a nicotinamide adenine dinucleotide can be a moiety of the 2'P phosphate 5 in a nicotinamide adenine dinucleotide phosphate molecule (see Figure 2 for location of the 2'P phosphate in nicotinamide adenine dinucleotide phosphate).

As used herein the term "sequence model" refers to a mathematical representation of the frequency and 10 order with which specific monomeric units or gaps occur in a set of polymers. The mathematical representation can include a probability of a given monomer occurring at a position in the sequence model. A probability of a given monomer occurring at a position in the sequence 15 model can be independent of other positions or can depend on the occupancy at any or all other positions in the sequence model. An example of a position independent sequence model is a Hidden Markov Model as described below. An example of a position dependent sequence model 20 is a sequence model with positions 1 through 10, where the occupancy at each position is modeled probabilistically. In a sequence model such as this, the probability that a specific monomer occurs at position 1 can vary based on the identity of the monomers that 25 occupy other positions such as 2, 8, and/or 9. A polymer included in the term can be, for example, a polypeptide or nucleotide. A sequence of a polypeptide that is useful in the methods of the invention can be represented by amino acids or nucleotides encoding amino acids of the 30 polypeptide such as codons. A sequence of a polypeptide that is useful in the methods of the invention includes a full sequence, or a portion thereof, including, for

example, a domain, region or residues separated by gaps in the full sequence.

As used herein the term "differential," when used in reference to sequence models, refers to a relationship between sequence models where a first sequence model represents a frequency with which specific monomeric units occur at a first set of positions in a polymer and a second sequence model represents the frequency with which specific monomeric units occur at a second set of positions in the same polymer. Sequence models that are differential with respect to each other can be produced from different subsets of monomeric units and/or have different parameters. For example, two sequence models that are differential with respect to each other can both be position dependent being produced from different training sets, position independent being produced from different training sets, one sequence model can be position dependent while another is position independent both being produced from the same training set or one sequence model can be position dependent while another is position independent each being produced from different training sets. Positions and frequencies can be represented redundantly in a first sequence model and second, differential sequence model so long as a set of positions or frequencies in the first model contains at least one position or frequency that is not present in the set of the differential model.

As used herein the term "relationship," when used in reference to a sequence and a sequence model, refers to a comparison of the presence, absence or identities of monomers at various positions in a polymer

sequence and sequence model. The term includes comparison of the presence, absence or identities of amino acids in a polypeptide sequence and a sequence model or comparison of the presence, absence or 5 identities of nucleotides in a polynucleotide sequence and a sequence model.

As used herein the term "correspondence," when used in reference to a sequence and a sequence model, refers to a statistically relevant similarity between the 10 sequence and the sequence model. A statistically relevant similarity can be indicated by a low expectation value (E value) or high bit score. The E value is understood in the art to be the statistically determined number of sequences that would be found by searching a 15 database with a random model that match as well or better to the random model than the sequence retrieved by searching the database with a trained model matches to the trained model, as described in Durbin et al., Biological Sequence Analysis Cambridge University Press 20 (1998). A sequence having a statistically relevant similarity to a sequence model can have an E value less than, or $-\ln(E)$ greater than, a cutoff E value. A cutoff E value can be at a specified threshold value of E including, for example, 100, 50, 10, 5, 2, 1, 0.5, 0.2, 25 0.1, or 0.01 that can be identified according to methods described below. The bit score is understood in the art to be a measure of the probability that the sequence belongs to the set of polypeptides used to train the model, as described in Durbin et al., *supra*.

30

As used herein the term "selected distance," when used in reference to a polypeptide, refers to a

- length separating locations in a polypeptide and/or separating locations in a polypeptide and bound ligand. A location in a polypeptide can include, for example, an amino acid location, an atom location, or location
- 5 identified relative to an amino acid such as a center of gravity or center of a volume occupied by the amino acid. A location in a bound ligand can include, for example, a moiety location, an atom location, or location identified relative to the bound ligand, or moiety thereof such as a
- 10 center of gravity or center of an occupied volume. A length separating two locations can be a length between points in a three dimensional structure including, for example, a length of a line drawn between locations in a high resolution structure model or a length measured by
- 15 spectroscopic means such as an NOE method. A length separating two locations can be a length between points in a primary sequence of a polypeptide including, for example, a number of amino acids separating two points, a number of atoms separating two points, or calculated
- 20 distances thereof based on theoretical bond lengths.
- Additionally, a selected distance can include a combination of lengths determined in a 3 dimensional structure and primary sequence. For example, amino acids within a selected distance can include a first subset of
- 25 those within an identified length from a bound ligand in the 3 dimensional structure and a second subset containing others within an identified number of amino acids, in the primary sequence, from those in the first subset.
- 30 The invention provides a method for identifying a pharmacocluster. The method includes the steps of (a) determining bound conformations of a ligand bound to

different polypeptides, and (b) clustering two or more bound conformations of the ligand having substantially the same bound conformation, thereby identifying a pharmacocluster. The invention also provides a method
5 for identifying a member of a pharmacocluster. The method includes the steps of (a) determining a bound conformation of a ligand bound to a polypeptide; and (b) determining a pharmacocluster having substantially the same bound conformation as the bound conformation,
10 thereby identifying the bound conformation of the ligand as a member of the pharmacocluster.

A bound conformation of a ligand bound to a polypeptide can be determined from a previously observed molecular structure or from data specifying a molecular
15 structure for a bound conformation of a ligand. Previously observed structures can be acquired for use in the invention by searching a database of existing structures. An example of a database that includes structures of bound conformations of ligands bound to
20 polypeptides is the Protein Data Bank (PDB, operated by the Research Collaboratory for Structural Bioinformatics, see Berman et al., Nucleic Acids Research, 28:235-242 (2000)). A database can be searched, for example, by querying based on chemical property information or on
25 structural information. In the latter approach, an algorithm based on finding a match to a template can be used as described, for example, in Martin, "Database Searching in Drug Design," J. Med. Chem. 35:2145-2154 (1992).

30 A bound conformation of a ligand bound to a polypeptide can be determined from an empirical

measurement, or from a database. Data specifying a structure can be acquired using any method available in the art for structural determination of a ligand bound to a polypeptide. For example, X-ray crystallography can be 5 performed with a crystallized complex of a polypeptide and ligand to determine a bound conformation of the ligand bound to the polypeptide. Methods for obtaining such crystal complexes and determining structures from them are well known in the art as described for example 10 in McRee et al., Practical Protein Crystallography, Academic Press, San Diego 1993; Stout and Jensen, X-ray Structure Determination: A practical guide, 2nd Ed. Wiley, New York (1989); and McPherson, The Preparation and Analysis of Protein Crystals, Wiley, New York (1982). 15 Another method useful for determining a bound conformation of a ligand bound to a polypeptide is Nuclear Magnetic Resonance (NMR). NMR methods are well known in the art and include those described for example in Reid, Protein NMR Techniques, Humana Press, Totowa NJ 20 (1997); and Cavanaugh et al., Protein NMR Spectroscopy: Principles and Practice, ch. 7, Academic Press, San Diego CA (1996).

A bound conformation of a ligand can also be determined from a hypothetical model. For example, a 25 hypothetical model of a bound conformation of a ligand can be produced using an algorithm which docks a ligand to a polypeptide of known structure and fits the ligand to the polypeptide binding site. Algorithms available in the art for fitting a ligand structure to a polypeptide 30 binding site include, for example, DOCK (Kuntz et al., J. Mol. Biol. 161:269-288 (1982)) and INSIGHT98 (Molecular Simulations Inc., San Diego, CA).

A molecular structure can be conveniently stored and manipulated using structural coordinates. Structural coordinates can occur in any format known in the art so long as the format can provide an accurate 5 reproduction of the observed structure. For example, crystal coordinates can occur in a variety of file types including, for example, .fin, .df, .phs, or .pdb as described for example in McRee, *supra*. Although the examples above describe structural coordinates derived 10 from X-ray crystallographic analysis or NMR spectroscopy, one skilled in the art will recognize that structural coordinates can be derived from any method known in the art to determine a bound conformation of a ligand bound to a polypeptide.

15 Structures at atomic level resolution can be useful in the methods of the invention. Resolution, when used to describe molecular structures, refers to the minimum distance that can be resolved in the observed structure. Thus, resolution where individual atoms can 20 be resolved is referred to in the art as atomic resolution. Resolution is commonly reported as a numerical value in units of Angstroms (\AA , 10^{-10} meter) correlated with the minimum distance which can be resolved such that smaller values indicate higher 25 resolution. Bound conformations of a ligand useful in the methods of the invention can have a resolution better than about 10 \AA , 5 \AA , 3 \AA , 2.5 \AA , 2.0 \AA , 1.5 \AA , 1.0 \AA , 0.8 \AA , 0.6 \AA , 0.4 \AA , or about 0.2 \AA or better. Resolution can also be reported as an all atom RMSD as 30 used, for example, in reporting NMR data. Bound conformations of a ligand useful in the methods of the invention can have an all atom RMSD better than about 10

Å, 5 Å, 3 Å, 2.5 Å, 2.0 Å, 1.5 Å, 1.0 Å, 0.8 Å, 0.6 Å, 0.4 Å, or about 0.2 Å or better.

An advantage of the methods of the invention is that a structure of a polypeptide bound to a bound conformation of a ligand need not be determined to identify a pharmacocluster. Thus, methods that detect only the structure of the ligand can be used in the invention. Additionally, in some cases determination or refinement of only the structure of the ligand in a polypeptide-ligand complex will be required. Methods that can be used to determine a conformation-dependent property of a ligand in a polypeptide-ligand complex without determining the structure of the polypeptide include, for example, Electron Nuclear Double Resonance spectroscopy (ENDOR, as described in Van Doorslaer and Schweiger, Naturwissenschaften 87:245-55(2000)), Electron Paramagnetic Resonance spectroscopy (EPR, described in Cantor and Schimmel Biophysical Chemistry, Part I: The conformation of biological macromolecules W. H. Freeman and Company (1980)), chemically induced dynamic nuclear polarization (CIDNP, described in Siebert et al., Glycoconj J. 14:945-9 (1997) and Consonni et al., FEBS Lett. 372:135-9 (1995)), solid state NMR (described in Mehring, M. High Resolution NMR spectroscopy in Solids, 2nd ed. Springer-Verlag, Berlin (1983) and liquid phase NMR (described in Wüthrich, NMR of Proteins and Nucleic Acids John Wiley & Sons, Inc. (1986)). Thus, the invention can be performed in a manner whereby the time and cost associated with a full determination of a polypeptide structure is avoided.

Any representation that correlates with the structure of a bound conformation of a ligand can be used in the methods of the invention. For example, a convenient and commonly used representation is a displayed image of the structure. Displayed images that are particularly useful for determining the bound conformation of a ligand bound to polypeptides include, for example, ball and stick models, density maps, space filling models, surface map, Connolly surfaces, Van der Waals surfaces or CPK model. Display of images as a computer output, for example, on a video screen can be advantageous as described below.

Clustering can be performed with any ligand or any number of bound conformations of a ligand. The methods of the invention can be performed by clustering 2 or more bound conformations of a ligand. For example, clustering can be performed with 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more or 20 or more bound conformations of a ligand. The methods of the invention can be used with any number bound conformations of a ligand. Due to the large sizes of data sets required to represent bound conformations of a ligand, methods of clustering bound conformations are generally performed on a computer. The methods are compatible with any computer that can support molecular modeling software including for example a personal computer, silicon graphics workstation, or supercomputer. A variety of computer software programs are available for molecular modeling including, for example, GRASP (Nicholls, A., *supra*), ALADDIN (Van Drie et al. *supra*), INSIGHT98 (Molecular Simulations Inc., San Diego CA),

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RASMOL (Sayle et al., Trends Biochem Sci. 20:374-376 (1995)) and MOLMOL (Koradi et al., J. Mol. Graphics 14:51-55 (1996)).

Once a bound conformation of a ligand bound to
5 different polypeptides has been determined, two or more
bound conformations of the ligand can be compared and
those having substantially the same bound conformation
can be clustered. Methods of comparison include, for
example, a method that provides alignment of two or more
10 bound conformations of a ligand and evaluation of the
degree of overlap in the two structures. Methods of
comparison can be performed in an iterative fashion until
a best fit is identified.

Methods of comparing bound conformations of
15 bound ligands include, for example, cluster analysis,
visual inspection and pairwise structural comparisons.
Cluster analysis is commonly performed by, but not
limited to, partitioning methods or hierarchical methods
as described, for example, in Kauffman and Rousseeuw,
20 Finding Groups in Data: An Introduction to Cluster
Analysis, John Wiley and Sons Inc., New York (1990).
Partitioning methods that can be used include, for
example, partitioning around mediods, clustering large
applications, and fuzzy analysis, as described in
25 Kauffman and Rousseeuw, *supra*. Hierarchical methods
useful in the invention include, for example,
agglomerative nesting, divisive analysis, and monothetic
analysis, as described in Kauffman and Rousseeuw, *supra*.
Algorithms for cluster analysis of molecular structures
30 are known in the art and include, for example, COMPARE
(Chiron Corp, 1995; distributed by Quantum Chemistry

program Exchange, Indianapolis IN). COMPARE can be used to make all possible pairwise comparisons between a set of conformations of the same ligand(s). COMPARE reads PDB files and uses a Ferro-Hermanns ORIENT algorithm for 5 a least squares root mean square (RMS) fit. The structures can be clustered into groups using the Jarvis-Patrick nearest neighbors algorithm. Based on the RMS deviation between ligand conformers, a list of 'nearest neighbors' for each conformer are generated. Two 10 conformers are then grouped together or clustered if: (1) the RMS deviation is sufficiently small and (2) if both conformers share a determined number of common 'neighbors'. Both criteria are adjusted by the program to generate clusters based on a user defined cutoff for 15 distance between individual clusters. Follow up analysis was conducted using InsightII to verify clusters. A member conformation is identified as being closer to the averaged coordinates of conformations within its family than to the averaged coordinates of any other family.

20 Using methods such as those described above, one skilled in the art will know how to identify conformations that are substantially the same. For example, similarity can be evaluated according to the goodness of fit between two or more bound conformations 25 of a ligand. Goodness of fit can be represented by a variety of parameters known in the art including, for example, the root mean square deviation (RMSD). A lower RMSD between structures correlates with a better fit compared to a higher RMSD between structures. Bound 30 conformations of a ligand having substantially the same conformations can be identified by comparing mean RMSD values within and between pharmacoclusters, for example,

as demonstrated in Example I. Accordingly, bound conformations of a ligand having substantially the same conformations can have a mean RMSD compared to an average structure for the pharmacocluster that is less than 1.1 Å. Two or more bound conformations of a ligand can be clustered by assigning bound conformations of a ligand into a collection such that the conformations of a ligand residing in the collection are substantially the same. Members of a pharmacocluster can also be identified as having RMSD values compared to an average structure for the pharmacocluster that are less than 1.0 Å, 0.9 Å, 0.8 Å, 0.7 Å, 0.6 Å, 0.5 Å, 0.4 Å, 0.3 Å, 0.2 Å or 0.1 Å.

A bound conformation of a ligand that is a member of a pharmacocluster can also be identified by comparing the RMSD for the bound conformation to an average conformation of the members in multiple pharmacoclusters. Using this value for comparison, a member conformation is identified as having a smaller RMSD when compared to the averaged coordinates of conformations within its family than when compared to the averaged coordinates of any other family. In addition, a member of a pharmacocluster can be identified as having an RMSD compared to an average conformation of the members in a pharmacocluster that is smaller than the RMSD between each family's average coordinates. For example, as described in Example I, RMSD values for members of pharmacoclusters 1-8 as presented in Tables 3A, 4A, 5A, 6A, 7A, 8A, 9A or 10A, respectively, can be compared to RMSD values between each pharmacocluster as presented in Table 2. Comparisons similar to those described above can be made for bound conformations of

any ligand according to the methods described in the Examples.

In addition, bound conformations of a ligand can be compared with respect to dihedral angles at 5 particular bonds. Exemplary methods for comparing dihedral angles between pharmacoclusters is described in Example I and Table 1. Comparison between dihedral angles can be used, for example, in combination with overall RMSD comparisons such as those described above. 10 Therefore, bound conformations that are not easily distinguished by comparison of overall RMSD alone, can be distinguished according to the combined comparison of RMSD and dihedral angle. Bound conformations of a ligand that are members of different pharmacoclusters can have 15 dihedral angles that differ, for example, by at least about 10 degrees, 30 degrees, 45 degrees, 90 degrees or 180 degrees.

The invention also provides a pharmacocluster selected from the cluster consisting of pharmacocluster 20 1, pharmacocluster 2, pharmacocluster 3, pharmacocluster 4, pharmacocluster 5, pharmacocluster 6, pharmacocluster 7, and pharmacocluster 8 correlated with the pharmacofamilies listed in Table 11.

Pharmacoclusters 1 through 8 contain bound 25 conformations of NAD(P)(H) determined from structures deposited in the PDB for NAD(P)(H) bound to oxidoreductase polypeptides. Pharmacoclusters are shown in Figure 1 and described in further detail in Example I. The pharmacoclusters of Figure 1 display substantial 30 overlap between bound conformations of NAD(P)(H) within

the cluster, as can be identified by visual inspection of the structures. Quantitative comparison of the bound conformations in each pharmacocluster demonstrates that each pharmacocluster displays less than about 1.1 Å difference in RMSD between each conformation of NAD(P)(H) and the average bound conformation for each cluster as described in Example I.

Pharmacoclusters can be used to identify a ligand having specificity for one or more polypeptide 10 pharmacofamilies (see Example V). As described herein, a pharmacophore model or conformer model can be derived from one or more cluster. These models can be used to identify a ligand having specificity for one or more pharmacofamilies of oxidoreductases, for example, by 15 using the model to query a database of molecules for a potential ligand or by using the model to guide in the design of a synthetic ligand. An example of using a pharmacophore of the invention to identify a binding compound is provided in Example VI.

20 Pharmacoclusters, including, for example, pharmacoclusters 1 through 8 can also be used to identify a new polypeptide member of a polypeptide pharmacofamily. Using the methods described herein, for example, a pharmacocluster can be used to produce a pharmacophore 25 model or conformer model to which a bound conformation of a ligand can be compared. A polypeptide bound to a bound conformation of a ligand that is similar to the model can be classified into an appropriate polypeptide pharmacofamily based on this comparison. By a similar 30 method, a bound conformation of a ligand can be directly compared to a pharmacocluster to classify the polypeptide

bound to the conformation of a ligand into an appropriate pharmacofamily.

The methods of the invention can also be used with a portion of a bound conformation of a ligand to

5 identify a pharmacocluster. The method consists of (a) determining a bound conformation of a ligand, or portion thereof, bound to two or more polypeptides, and (b) clustering two or more bound conformations of the ligand, or portion thereof having substantially the same bound

10 conformation, thereby identifying a pharmacocluster.

A bound conformation of a portion of a ligand can include selected atoms and/or bonds of a ligand and can include, for example, a continuous sequence of atoms and/or bonds or a discontinuous sequence of selected

15 atoms and/or bonds that, when described independent of the complete ligand structure, may not appear to be attached to each other. Such a portion can include 2 or more atoms of a bound conformation of a ligand or 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or

20 more, 9 or more, 10 or more, 15 or more, 20 or more, 25 or more or 50 or more atoms of a bound conformation of a ligand. A bound conformation of a portion of a ligand bound to a polypeptide can be identified according to the same methods described above for identifying a bound

25 conformation of a ligand bound to a polypeptide. Two or more bound conformations of a portion of a ligand can be clustered as described above so long as the bound conformations that are clustered correspond to bound portions of the ligand having the same structural

30 formula. For example, in a case where determination of the complete structure of a ligand has not been achieved,

a bound conformation of a portion of the ligand corresponding to the structurally determined portion can be used in the methods of the invention.

A pharmacocluster can include portions of bound conformations derived from different ligands so long as the portions have a core bound conformation that is substantially the same. For example, portions having the same structural formula and bond configuration can share a core bound conformation. The bond configuration describes the relative position of atoms attached to a chiral atom of a ligand. Accordingly, R and S stereoisomers of a chiral atom have different bond configurations. Other terms used in the art to designate different bond configurations include, for example, cis and trans configurations of atoms attached to carbons that are double bonded, or Z and E configurations of atoms attached to carbons that are double bonded. An example of portions of ligands having the same structural formula and bond configuration that can share a core bound conformation are the nicotinamide adenine dinucleotide portions of nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD). Additionally, portions of ligands having different charge, atom substitution or bond hybridization can share a core bound conformation. An example of portions of ligands having different charge and bond hybridization that can share a core bound conformation are the nicotinamide adenine dinucleotide portions of oxidized nicotinamide adenine dinucleotide (NAD) and reduced nicotinamide adenine dinucleotide (NADH). In cases where the core structures of two ligands bind with substantially the same conformation to

polypeptides, the core bound conformations can be clustered according to the methods of the invention (see Example I).

Substantially the same bound conformation of a portion of a bound conformation of a ligand, including non-continuous atoms, can be identified according to the root mean square deviation and compared directly. Conformations of portions having different numbers of atoms can also be compared via root mean square deviation per equivalent atom (RMSD/N , where N is the number of atoms compared). A lower value of RMSD/N indicates increased similarity between the two or more bound ligand conformations that are clustered. One skilled in the art will know that RMSD/N has a compensational origin and consideration of the effect of N is required for comparison of RMSD/N between pharmacoclusters having different values of N . For example, the lower the value of RMSD/N the lower should be the value of N to indicate substantial similarity.

The invention can be used with any ligand for which bound conformations of the ligand bound to different polypeptides can be determined including, for example, chemical or biological molecules such as simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, peptidomimetics, carbohydrates, lipids, nucleic acids, and the like.

In one embodiment, the compositions and methods of the invention can be used with a ligand that is a nucleotide derivative including, for example, a nicotinamide adenine dinucleotide-related molecule.

Nicotinamide adenine dinucleotide-related (NAD-related) molecules that can be used in the methods of the invention can be selected from the group consisting of oxidized nicotinamide adenine dinucleotide (NAD^+),
5 reduced nicotinamide adenine dinucleotide (NADH), oxidized nicotinamide adenine dinucleotide phosphate (NADP^+), and reduced nicotinamide adenine dinucleotide phosphate (NADPH). An NAD-related molecule can also be a mimetic of the above-described molecules. Use of a NAD-
10 related molecule to identify pharmacoclusters is described in Example I.

A mimetic is a molecule that has at least one function that is substantially the same as a function of a second molecule. A mimetic of a ligand can be
15 identified according to its ability to bind to the same sites on a polypeptide as the ligand. For example, a mimetic can be identified by a binding competition assay using a ligand and a mimetic. The structure of a mimetic can be similar or different compared to the structure of
20 the second molecule. The term can encompass molecules having portions similar to corresponding portions of the ligand in terms of structure or function.

Examples of mimetics to the common ligand NADH, for example cibacron blue, are described in Dye-Ligand Chromatography, Amicon Corp., Lexington MA (1980).
25 Numerous other examples of NADH-mimics, including useful modifications to obtain such mimics, are described in Everse et al. (eds.), The Pyridine Nucleotide Coenzymes, Academic Press, New York NY (1982). Particular analogs
30 include nicotinamide 2-aminopurine dinucleotide, nicotinamide 1-nicotinamide 8-azidoadenine dinucleotide, nicotinamide 1-

deazapurine dinucleotide, 3-aminopyridine adenine dinucleotide, 3-acetyl pyridine adenine dinucleotide, thiazole amide adenine dinucleotide, 3-diazoacetylpyridine adenine dinucleotide and 5-5 aminonicotinamide adenine dinucleotide. Particular mimetics can be identified and selected by ligand-displacement assays, for example using competitive binding assays with a known ligand as is well known in the art. Mimetic candidates can also be identified by 10 searching databases of compounds for structural similarity with the common ligand or a mimetic.

In another embodiment, the methods of the invention can be used with a ligand that is an adenosine phosphate-related molecule. Adenosine phosphate-related 15 molecules can be selected from the group consisting of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and cyclic adenosine monophosphate (cAMP). An adenosine phosphate-related molecule can also be a mimetic of the above- 20 described molecules. A mimetic of an adenosine phosphate-related molecule that can be used in the invention includes, for example, quercetin, adenylylimidodiphosphate (AMP-PNP) or olomoucine.

A ligand useful in the methods of the invention 25 can be a cofactor, coenzyme or vitamin including, for example, NAD, NADP, or ATP as described above. Other examples include thiamine (vitamin B₁), riboflavin (vitamin B₂), pyridoximine (vitamin B₆), cobalamin (vitamin B₁₂), pyrophosphate, flavin adenine dinucleotide 30 (FAD), flavin mononucleotide (FMN), pyridoxal phosphate, coenzyme A, ascorbate (vitamin C), niacin, biotin, heme,

porphyrin, folate, tetrahydrofolate, nucleotide such as guanosine triphosphate, cytidine triphosphate, thymidine triphosphate, uridine triphosphate, retinol (vitamin A), calciferol (vitamin D₂), ubiquinone, ubiquitin, α -tocopherol (vitamin E), farnesyl, geranylgeranyl, pterin, pteridine or S-adenosyl methionine (SAM).

A polypeptide can be used as a ligand in the invention. For example, a ligand can be a naturally occurring polypeptide ligand such as a ubiquitin or 10 polypeptide hormone including, for example, insulin, human growth hormone, thyrotropin releasing hormone, adrenocorticotrophic hormone, parathyroid hormone, follicle stimulating hormone, thyroid stimulating hormone, luteinizing hormone, human chorionic 15 gonadotropin, epidermal growth factor, nerve growth factor and the like. In addition a polypeptide ligand can be a non-naturally occurring polypeptide that has binding activity. Such polypeptide ligands can be identified, for example, by screening a synthetic 20 polypeptide library such as a phage display library or combinatorial polypeptide library as described below. A polypeptide ligand can also contain amino acid analogs or derivatives such as those described below. Methods of isolation of a polypeptide ligand are well known in the 25 art and are described, for example, in Scopes, Protein Purification: Principles and Practice, 3rd Ed., Springer-Verlag, New York (1994); Duetscher, Methods in Enzymology, Vol 182, Academic Press, San Diego (1990); and Coligan et al., Current protocols in Protein Science, 30 John Wiley and Sons, Baltimore, MD (2000).

A nucleic acid can also be used as a ligand in the invention. Examples of nucleic acid ligands useful in the invention include DNA, such as genomic DNA or cDNA or RNA such as mRNA, ribosomal RNA or tRNA. A nucleic acid ligand can also be a synthetic oligonucleotide. Such ligands can be identified by screening a random oligonucleotide library for ligand binding activity, for example, as described below. Nucleic acid ligands can also be isolated from a natural source or produced in a recombinant system using well known methods in the art including, for example, those described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, New York (1989); Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999).

A ligand used in the invention can be an amino acid, amino acid analog or derivatized amino acid. An amino acid ligand can be one of the 20 essential amino acids or any other amino acid isolated from a natural source. Amino acid analogs useful in the invention include, for example, neurotransmitters such as gamma amino butyric acid, serotonin, dopamine, or norepinephrine or hormones such as thyroxine, epinephrine or melatonin. A synthetic amino acid, or analog thereof, can also be used in the invention. A synthetic amino acid can include chemical modifications of an amino acid such as alkylation, acylation, carbamylation, iodination, or any modification that derivatizes the amino acid. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl

groups, chloroacetyl groups or formyl groups. Free carboxyl groups can be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups can be derivatized to form O-acyl or 5 O-alkyl derivatives. The imidazole nitrogen of histidine can be derivatized to form N-im-benzylhistidine. Naturally occurring amino acid derivatives of the twenty standard amino acids can also be included in a cluster of bound conformations including, for example, 10 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, homoserine, ornithine or carboxyglutamate.

A lipid ligand can also be used in the invention. Examples of lipid ligands include triglycerides, phospholipids, glycolipids or steroids. 15 Steroids useful in the invention include, for example, glucocorticoids, mineralocorticoids, androgens, estrogens or progestins.

Another type of ligand that can be used in the invention is a carbohydrate. A carbohydrate ligand can 20 be a monosaccharide such as glucose, fructose, ribose, glyceraldehyde, or erythrose; a disaccharide such as lactose, sucrose, or maltose; oligosaccharide such as those recognized by lectins such as agglutinin, peanut lectin or phytohemagglutinin, or a polysaccharide such as 25 cellulose, chitin, or glycogen.

Methods for producing pluralities of compounds to use as ligands, including chemical or biological molecules such as simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, 30 peptidomimetics, carbohydrates, lipids, nucleic acids,

and the like, are well known in the art (see, for example, in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers.

- 5 3:75-94 (1998); Eichler et al., Med. Res. Rev. 15:481-496 (1995); Gordon et al., J. Med. Chem. 37: 1233-1251 (1994); Gordon et al., J. Med. Chem. 37: 1385-1401 (1994); Gordon et al., Acc. Chem. Res. 29:144-154 (1996); Wilson and Czarnik, eds., Combinatorial Chemistry:
- 10 Synthesis and Application, John Wiley & Sons, New York (1997), Gold et al., U.S. Pat Nos. 5,475,096 (1995), 5,789,157 (1998), and 5,270,163 (1993)). The advantage of using such a combinatorial library is that molecules do not have to be individually generated to identify a
- 15 ligand that binds a polypeptide. Also, no prior knowledge of the exact characteristics of a binding polypeptide is required when using a combinatorial library. Libraries containing large numbers of natural and synthetic compounds also can be individually
- 20 synthesized or obtained from commercial sources.

In addition, the invention provides a method for identifying a conformation-dependent property of a ligand. The method includes the steps of (a) determining bound conformations of a ligand bound to different polypeptides; (b) identifying two or more bound conformations of the ligand having substantially the same bound conformation, and (c) identifying a conformation-dependent property of the bound conformations of the ligand having substantially the same bound conformation, the conformation-dependent property being correlated with the bound conformation of the ligand.

A conformation-dependent property can be identified as any property that correlates with a bound conformation of a ligand such that a change in the bound conformation results in a change in the conformation-dependent property. Accordingly, a bound conformation of a ligand, or a portion thereof, can be a conformation-dependent property. A portion of a bound conformation of a ligand can be a contiguous fragment or a non-contiguous set of atoms or bonds. A bound conformation of a ligand, or portion thereof, can be identified by any method for determining the three dimensional structure of a ligand including as disclosed herein.

Other conformation-dependent properties include, for example, absorption and emission of heat, absorption and emission of electromagnetic radiation, rotation of polarized light, magnetic moment, spin state of electrons, or polarity, as disclosed herein, or other properties that can be identified as a spectroscopic signal. Methods known in the art for measuring changes in absorption and emission of heat that correlate with changes in bound conformation of a ligand include, for example, calorimetry. Methods known in the art for measuring changes in absorption and emission of electromagnetic radiation as they correlate with changes in bound conformation of a ligand include, for example, UV/VIS spectroscopy, fluorimetry, luminometry, infrared spectroscopy, Raman spectroscopy, resonance Raman spectroscopy, X-ray absorption fine structure spectroscopy (XAFS) and the like. A change in a bound conformation of a ligand that is correlated with a change in rotation of polarized light can be measured with circular dichroism spectroscopy or optical rotation

spectroscopy. A change in magnetic moment or spin state of an electron that correlates with a change in a bound conformation can be measured, for example, with Electron paramagnetic resonance spectroscopy (EPR) or nuclear magnetic resonance spectroscopy (NMR).

When based on NMR data, a conformation-dependent property can be identified as an NMR signal including, for example, chemical shift, *J* coupling, dipolar coupling, cross-correlation, nuclear spin relaxation, transferred nuclear Overhauser effect, and any combination thereof. A conformation-dependent property can be identified by NMR methods in both fast and slow exchange regimes. For example, in many cases, the exchange rate of a complex between ligand and polypeptide is faster than the ligand spin relaxation rate ($1/T_{1H}$). In this situation, referred to as the "fast exchange regime," transferred nuclear Overhauser effect (NOE) experiments can be performed to measure an intra-ligand proton-proton distance (Wuthrich, NMR of proteins and Nucleic Acids, Wiley, New York (1986) and Gronenborn, J. Magn. Res. 53:423-442 (1983)). Labeling of polypeptides is not required, and the ligand polypeptide concentration ratio can be adjusted to minimize line broadening of the ligand resonances while retaining strong NOE contribution from the bound form.

In a fast exchange regime, cross-correlated relaxation measurements can also provide structural information on ligand torsion angles (Carlomagno et al., J. Am. Chem Soc. 121:1945-1948 (1999)). These measurements include the ^1H - ^1H dipole-dipole cross-correlation but can be extended to other cross-correlated

relaxation mechanisms involving also homo- and heteronuclear chemical shielding anisotropy relaxation, as well as quadrupolar relaxation. For most of these heteronuclear experiments, the natural abundance of the
5 isotope can be exploited. In cases where natural abundance of the isotope measured is not sufficient, isotope enriched ligands can be obtained from commercial sources such as Isotek (Miamisburg, OH) or Cambridge Isotope Laboratories (Andover, MA) or prepared by methods
10 known in the art. Another method to determine a conformation-dependent property of a ligand in a fast exchange regime is use of residual homo- and heteronuclear dipolar couplings in partially aligned samples (Tolman et al. Proc. Natl. Acad. Sci. USA
15 92:9279-9283 (1995)).

In the slow exchange regime, the NMR signals arising from the bound conformation of the ligand are distinguished from those of the polypeptide to reduce resonance overlap. This can be achieved with different
20 isotope labeling schemes of polypeptide, ligand or both. For large systems, perdeuteration of macromolecules and TROSY-type experiments (Pervushkin, Proc. Natl. Acad.
Sci. USA 94:12366-12371 (1997)) can be used to minimize signal losses due to fast transverse relaxation of the
25 resonances of the complex. With the appropriate sample requirements and isotope filtered experiments, cross-correlations, cross-relaxations and residual dipolar couplings can be measured and provide necessary structural information.

30 In addition, homo- and heteronuclear two and three bond J couplings can be obtained to provide

information on torsion angles (Wuthrich, *supra*). For example, as shown in Table 1 the bound conformations of NADP in pharmacocluster 4 and pharmacocluster 5 differ by a torsion angle defined by the atoms PN-O5'N-C5'N-C4'N
5 (See Figure 2 for atom labeling and bond location). Specifically, pharmacocluster 4 has a PN-O5'N-C5'N-C4'N torsion angle of 145 degrees and pharmacocluster 5 has a PN-O5'N-C5'N-C4'N angle of -112 degrees. These torsion angles can be measured and distinguished by measuring the
10 three bond $^{31}\text{P}-^{13}\text{C}4'$ J coupling constants that correspond to this torsion angle (Marino, Acc. Chem. Res. 32:614-623 (1999)). Basically, two $^1\text{H}-^{13}\text{C}$ correlation experiments can be performed with and without ^{31}P decoupling during ^{13}C evolution. The intensity ratio of the $^1\text{H} 4'/^{13}\text{C}4'$ cross
15 peak from each experiment is proportional to the $^{31}\text{P}-^{13}\text{C}4'$ J coupling constant.

Correlation of a conformation-dependent property with a bound conformation of a ligand can be achieved by any method that has sufficient sensitivity to
20 detect changes that correlate with changes in bound conformation of a ligand. Such a correlation can be determined by measuring a conformation-dependent property for various conformations of a ligand and determining the extent of change in the signal with change in the
25 conformation. Signal changes that correlate with changes in conformation and that are detectable with a signal to noise ratio accepted in the art as significant can be used in the invention.

Correlation between a conformation-dependent property and a conformation can be determined for a
30 ligand bound to any partner so long as binding is

specific and stable. For example, for purposes of establishing a correlation, changes in a conformation dependent property that correlate with changes in bound conformation of a ligand can be determined for a ligand
5 bound to polypeptides from different polypeptide pharmacofamilies. A bound conformation of the ligand in each complex can be determined and a conformation-dependent property can be measured for each complex.

Comparison of bound conformations of the ligand in each
10 complex with a measured conformation-dependent property can be used to establish a correlation. Demonstration of a method for establishing a correlation between an NMR signal and bound conformations of a ligand is described herein (see Example IV). Other methods for correlating
15 spectroscopic signals with bound conformations of a ligand are known in the art including, for example, correlation of transferred NOE signals with *anti* and *syn* conformations of the nicotinamide ring in NADPH as described in Sem and Kasper Biochemistry 31:3391-3398
20 (1992). Correlation of transferred NOE signals with conformation is also described in Clore and Gronenborn, J. Magn. Reson. 48:402-417 (1982).

A correlation between a bound conformation and a conformation-dependent property can also be established
25 for a ligand bound to a non-polypeptide binding partner because a conformation-dependent property of a ligand can be independent of interactions that differ between binding partners so long as the ligand is in the same bound conformation when bound to the binding partners.
30 Other binding partners include, for example, nucleic acids, carbohydrates, and synthetic organometallic complexes.

A method of the invention for identifying a conformation-dependent property of a ligand can also include the steps of (a) determining a bound conformation of a ligand, or portion thereof, bound to two or more polypeptides; (b) identifying two or more bound conformations of the ligand, or portion thereof, having substantially the same bound conformation, and (c) identifying a conformation-dependent property of the bound conformations of the ligand, or portion thereof, 5 having substantially the same bound conformation, the conformation-dependent property being correlated with the bound conformation of the ligand, or portion thereof. A conformation-dependent property of a portion of a ligand can be identified, for example, by using the methods 10 described above for identifying a conformation-dependent property of a ligand.

The invention also provides a method for identifying a polypeptide pharmacofamily. The method includes the steps of (a) determining bound conformations 20 of a ligand bound to different polypeptides of a polypeptide family, and (b) identifying two or more bound conformations of the ligand having substantially different bound conformations, thereby identifying at least two polypeptide pharmacofamilies exhibiting binding 25 specificity for the two or more substantially different bound conformations of the ligand.

A method for identifying a polypeptide pharmacofamily can include the steps of (a) determining bound conformations of a ligand bound to different polypeptides of a polypeptide family; (b) clustering 30 bound conformations of a ligand having substantially the

same conformations into pharmacoclusters; and (c) identifying a first polypeptide that binds a bound conformation of a ligand in one pharmacocluster and a second polypeptide that binds a bound conformation of a 5 ligand in a second pharmacocluster as belonging to separate polypeptide pharmacofamilies.

Polypeptides of a polypeptide family can be identified by their ability to specifically bind to the same ligand, or portion thereof. Specific binding 10 between a polypeptide and a ligand can be identified by methods known in the art. Methods of determining specific binding include, for example, equilibrium binding analysis, competition assays, and kinetic assays as described in Segel, Enzyme Kinetics John Wiley and Sons, New York (1975), and Kyte, Mechanism in Protein Chemistry Garland Pub. (1995). Thermodynamic and kinetic constants can be used to identify and compare 15 polypeptides and ligands that specifically bind each other and include, for example, dissociation constant (K_d), association constant (K_a), Michaelis constant (K_m), inhibitor dissociation constant (K_{is}) association rate constant (k_{on}) or dissociation rate constant (k_{off}). For 20 example, a family can be identified as having members that can specifically bind a ligand with a K_d of at most 25 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M or lower.

A family of polypeptides that bind a ligand can contain a pharmacofamily that binds substantially the same conformation of the ligand, or portion thereof. The 30 methods can be used to identify any number of pharmacofamilies in a family according to the number of

different bound conformations of a ligand identified. In cases where two or more polypeptide pharmacofamilies reside in a polypeptide family, the pharmacofamilies can be distinguished according to differences in bound 5 conformations of a ligand bound to the polypeptides. In this case, a bound conformation of a ligand can be determined and compared according to the methods described herein. Polypeptides bound to different bound conformations of a ligand can be identified as those that 10 do not show substantial overlap of all corresponding atoms when bound conformations are overlaid. Thus, polypeptides that bind different bound conformations of a ligand can be separated into different pharmacofamilies. Pharmacofamilies in turn can be identified as containing 15 polypeptides that bind substantially the same bound conformation of a ligand (see Examples II and III).

A pharmacofamily of polypeptides identified by the methods of the invention can have additional similarities that correlate with similarities in bound 20 conformation of a ligand. For example, a polypeptide pharmacofamily identified by the methods of the invention can consist of polypeptide members that share characteristics that are unique to the pharmacofamily when compared to one or more other polypeptides in a 25 different pharmacofamily of the same family. Such characteristics can include, for example, protein fold, evolutionary relatedness, enzymatic activity, domain structure, subcellular localization, interaction partners, or participation in a similar metabolic or 30 signal transduction pathway. A demonstration of a correlation between ligand bound conformation and another characteristic of polypeptides in a pharmacofamily is

provided in Example II, which describes correlation of bound conformation of a ligand with polypeptide structure.

- An example of a polypeptide family having
- 5 multiple pharmacofamilies that can be identified by the methods of the invention includes NAD(P)(H) binding polypeptides. Polypeptide pharmacofamilies identified according to differences in bound conformations of NAD(P)(H) are described in Example II and Table 11.
- 10 Thus, the methods can be used to identify a polypeptide pharmacofamily selected from the group consisting of pharmacofamily 1, pharmacofamily 2, pharmacofamily 3, pharmacofamily 4, pharmacofamily 5, pharmacofamily 6, pharmacofamily 7, and pharmacofamily 8.

- 15 The invention provides a polypeptide pharmacofamily, comprising polypeptides that bind to substantially the same bound conformation of a nicotinamide adenine dinucleotide-related molecule selected from pharmacofamily 1, pharmacofamily 2,
- 20 pharmacofamily 3, pharmacofamily 4, pharmacofamily 5, pharmacofamily 6, pharmacofamily 7, and pharmacofamily 8 as listed in Table 11.

- Pharmacofamilies 1 through 8 consist of the polypeptide members provided in Table 11 (see Example
- 25 III). The polypeptides in pharmacofamily 1 have the NAD(P)(H) binding Rossman fold in common, are all in the NAD(P)(H) binding Rossman SCOP Superfamily, and fall into the SCOP families of the amino-terminal domain of glyceraldehyde-3-phosphate dehydrogenase, the carboxy-
- 30 terminal domain of alcohol/glucose dehydrogenase, the NAD

binding domain of formate/glycerate dehydrogenase, the carboxy-terminal domain of amino acid dehydrogenase, or the amino-terminal domain of lactate & malate dehydrogenase.

5 The polypeptides in pharmacofamily 2 have the NAD(P)(H) binding Rossmann fold in common, are all in the NAD(P)(H) binding Rossmann SCOP Superfamily, and fall into the SCOP families of the carboxy-terminal domain of amino acid dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase.

The polypeptides in pharmacofamily 3 have the NAD(P)(H) binding Rossmann fold in common, are all in the NAD(P)(H) binding Rossmann SCOP Superfamily, and fall into the tyrosine-dependent oxidoreductase SCOP family.

15 The polypeptides in pharmacofamily 4 have the heme-linked catalase fold and are in the heme-linked catalase SCOP superfamily and heme-linked catalase SCOP family.

20 The polypeptides in pharmacofamily 5 have the β - α TIM barrel fold in common, are all in the NAD(P)(H) linked oxidoreductase SCOP Superfamily, and fall into the aldo-keto reductase SCOP family.

25 The polypeptides in pharmacofamily 6 are dihydrofolate reductases that all show the dihydrofolate reductase fold and fall into the dihydrofolate reductase SCOP superfamily and family.

The polypeptides in pharmacofamily 7 have the FAD/NAD(P)(H) binding domain fold in common, are all in the FAD/NAD(P)(H) binding domain SCOP Superfamily, and fall into the the amino-terminal and central domains of 5 FAD/NAD linked reductase SCOP family.

The polypeptides in pharmacofamily 8 have the ferrodoxin like fold in common, are all in the ferrodoxin like SCOP Superfamily, and fall into the NADPH-cytochrome P450 reductase or reductase SCOP families.

10 Polypeptide pharmacofamilies 1 through 8 were identified according to binding interactions with bound conformations of NAD(P)(H) in pharmacoclusters 1 through 8, as described in Example II. Accordingly, the invention provides a polypeptide pharmacofamily, 15 comprising polypeptides that bind to a nicotinamide adenine dinucleotide-related molecule having a bound conformation selected from pharmacocluster 1, pharmacocluster 2, pharmacocluster 3, pharmacocluster 4, pharmacocluster 5, pharmacocluster 6, pharmacocluster 7, 20 and pharmacocluster 8.

The invention additionally provides a method for identifying a member of a polypeptide pharmacofamily. The method consists of (a) determining a conformation-dependent property of a ligand bound to a polypeptide, 25 and (b) determining a pharmacocluster having substantially the same conformation-dependent property as the conformation-dependent property determined for the bound ligand, wherein a polypeptide pharmacofamily binds 30 the ligand in a conformation of the pharmacocluster, thereby identifying the polypeptide as a member of the

polypeptide pharmacofamily. For example, the method can be used with a ligand such as a nicotinamide adenine dinucleotide-related molecule or adenosine phosphate-related molecule (see Examples II and III).

5 The methods of the invention allow a new member of a polypeptide pharmacofamily to be identified based on correlation of a conformation-dependent property of a bound conformation of a ligand bound to a polypeptide with a conformation-dependent property established for a
10 bound conformation of the ligand bound to another polypeptide in the same pharmacofamily. Thus, a classification can be made based on ligand structure without requiring determination of the bound conformation of the ligand. In one embodiment, the conformation-
15 dependent property can be a model of a bound conformation. A bound conformation of a ligand bound to a test polypeptide can be determined, and the bound conformation can be compared to a pharmacocluster according to the methods described herein. Substantial
20 overlap between the bound conformation of the ligand bound to the test polypeptide and another bound conformation of the ligand bound to a polypeptide in a pharmacofamily can be used to identify the test polypeptide as a member of that polypeptide
25 pharmacofamily.

In another embodiment, the conformation-dependent property can be a spectroscopic signal that is correlated with the conformation of a ligand. A spectroscopic signal can be measured for the ligand bound
30 to a test polypeptide. The signal can be compared to a signal correlated with a bound conformation of a ligand

bound to a polypeptide in a polypeptide pharmacofamily. Substantial similarity between the two signals indicates that the bound conformation of the ligand bound to the test polypeptide is substantially similar to the bound 5 conformation of the ligand bound to the polypeptides of the pharmacofamily. Thus, the test polypeptide can be identified as a member of the polypeptide pharmacofamily.

The invention provides rapid and efficient 10 methods that can be used in a high-throughput screening format. High-throughput methods can be useful for identifying a member of a polypeptide pharmacofamily. In a case where a conformation-dependent property can be rapidly detected and processed, automated methods can be 15 created for measuring samples in rapid succession or measuring multiple samples in parallel. Automated methods can be used for rapidly handling samples including, for example, robotic instruments. A combination of automated sample handling methods with 20 detection of a conformation-dependent property can, therefore, be useful in a high-throughput screening method.

According to the methods of the invention a compound can be identified that has greater specificity 25 for the polypeptides of one pharmacofamily than for other polypeptides in the same family. Such a compound can be used to identify new members of a pharmacofamily using a binding assay. For example, a mimetic or analog of a ligand can be identified that preferentially adopts a 30 conformation more similar to conformations in a particular pharmacocluster than those in other

pharmacoclusters. Such a mimetic or analog can be used in a any binding assay capable of detecting interactions with a polypeptide, including, for example, high-throughput methods.

5 A member of a polypeptide pharmacofamily can
also be identified by searching a database of bound
conformations of a ligand. For example, a bound
conformation of a ligand that binds to a polypeptide of
an identified pharmacofamily can be used as a query in a
10 3 dimensional search of a database containing bound
conformations of a ligand. Overlap between the query
conformation and a retrieved bound conformation of the
ligand can be used to identify a polypeptide bound to the
retrieved bound conformation of the ligand as a member of
15 the same polypeptide pharmacofamily as a polypeptide that
binds the query bound conformation (see Example I).

The invention also provides a method of modeling the three dimensional structure of a polypeptide. The method consists of (a) determining a 20 conformation-dependent property of a ligand bound to a polypeptide; (b) determining a pharmacocluster having substantially the same conformation-dependent property as the conformation-dependent property determined for the bound ligand, wherein a polypeptide pharmacofamily binds 25 the ligand in a conformation of the pharmacocluster, thereby identifying the polypeptide as a member of the polypeptide pharmacofamily, and (c) modeling the three dimensional structure of the polypeptide according to a structural model of the second member of the polypeptide 30 pharmacofamily.

As disclosed herein, polypeptides in a pharmacofamily can have similar characteristics including, for example, similar 3 dimensional structure. Therefore, the 3 dimensional structure of a polypeptide identified by the invention as a member of a pharmacofamily can be modeled using a polypeptide that is in the same pharmacofamily and for which the structure is known. A variety of methods are known in the art for modeling the three dimensional structure of a polypeptide according to the amino acid sequence of the polypeptide and a structure of a second polypeptide used as a template. Available algorithms include, for example, GRASP (Nicholls, A., *supra*), ALADDIN (Van Drie et al. *supra*), INSIGHT98 (Molecular Simulations Inc., San Diego CA), RASMOL (Sayle et al., Trends Biochem Sci. 20:374-376 (1995)) and MOLMOL (Koradi et al., J. Mol. Graphics 14:51-55 (1996)).

A model of a polypeptide determined by the methods of the invention can be useful for identifying a function of the polypeptide. For example, residues of a polypeptide that are involved in binding can be identified using a model of the invention. Residues identified as participating in binding can be modified, for example, to engineer new functions into a polypeptide, to reduce an intrinsic activity of a polypeptide, or to enhance an intrinsic activity of a polypeptide. In another example, a model of a polypeptide can be compared to other polypeptide structures to identify similar functions. Exemplary functions that can be identified from a polypeptide structure include binding interactions with other polypeptides and catalytic activities.

The invention also provides a method for constructing a ligand conformer model by determining an average structure of the bound conformations of a ligand in a pharmacocluster. A method for constructing a ligand 5 conformer model can include the steps of (a) determining bound conformations of a ligand bound to different polypeptides; (b) clustering two or more bound conformations of the ligand having substantially the same bound conformation, thereby identifying a 10 pharmacocluster, and (c) determining an average structure of the bound conformations of the ligand in the pharmacocluster. Additionally, a method for constructing a ligand conformer model can include the steps of (a) determining a bound conformation of a ligand bound to a 15 polypeptide; (b) determining a pharmacocluster having substantially the same bound conformation as the bound conformation, thereby identifying the bound conformation of the ligand as a member of the pharmacocluster, and (c) determining an average structure of the bound 20 conformations of the ligand in the pharmacocluster.

An average structure of the bound conformations of a ligand in a pharmacocluster can be determined by a variety of methods known in the art. For example, an average structure can be determined by overlaying bound 25 conformations, or portions thereof, and identifying an average location for each atom. Bound conformations in a group to be averaged can be overlaid relative to a single member or relative to a centroid position for each atom. Algorithms for determining an average structure 30 are known in the art and include for example the OVERLAY routine in INSIGHT98 (Molecular Simulations Inc., San Diego CA).

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The format of a ligand conformer model can be chosen based on the method used to generate the model and the desired use of the model. In this regard, a conformer model can be represented as a single structure.

- 5 The resulting structure can be a unique structure compared to the conformations in the pharmacocluster from which it was derived. Thus, the conformer model can be a new structure never before observed in nature. A model represented by a single structure can be useful for
- 10 making visual comparisons by overlaying other structures with the model. A conformer model can also be represented as a plurality of structures incorporating all or a subset of the bound conformations in the pharmacocluster. A model represented by multiple
- 15 structures can be useful for identifying a range of minor deviations in the model.

- In yet another representation, the conformer model can be a volume surrounding all or a subset of the bound conformations in the pharmacocluster. A model showing volume can be useful for comparing other structures in a fitting format such that a structure which fits within the volume of the model can be identified as substantially similar to the model. One approach that can be used to fit a structure to a volume
- 20 is comparison of equivalent surface patches using gnomonic projection as described for example in Chau and Dean, J. Mol. Graphics 5:97 (1987). Use of a gnomonic projection to compare structures is also described in Doucet and Weber, Computer-Aided Molecular Design: Theory
- 25 and Applications, Academic Press, San Diego CA (1996).
- 30 Algorithms which can be used to fit a structure to a volume are known in the art and include, for example,

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CATALYST (Molecular Simulations Inc., San Diego, CA) and THREEDOM which is a part of the INTERCHEM package which makes use of an Icosahedral Matching Algorithm (Bladon, *J. Mol. Graphics* 7:130 (1989) for the comparison and 5 alignment of structures. An exemplary method of identifying a binding compound by searching a database of structures using a gnomonic projection is provided in Example V.

10 A conformer model can be useful in querying a database of polypeptide structures to find other members of a polypeptide pharmacofamily. For example, a member of a polypeptide pharmacofamily can be identified by querying a database of bound conformations of a ligand to 15 identify a retrieved bound conformation of a ligand that is substantially similar to the query structure, thereby identifying a polypeptide bound to the retrieved bound conformation as a member of the same pharmacofamily as a polypeptide bound to the query bound conformation. A 20 conformer model can also be used to identify a new member of a polypeptide pharmacofamily by querying a database of one or more polypeptide structures using an algorithm that docks the conformer model, wherein a favorable docking result with a retrieved polypeptide indicates 25 that the retrieved polypeptide is a member of the same polypeptide pharmacofamily as a polypeptide bound to the bound conformation used as a query. In the latter mode, a potential new member of a pharmacofamily from which the conformer model was derived can be identified. The 30 database queries described above can be performed with algorithms available in the art including, for example, THREEDOM and CATALYST.

An advantage of the invention is that a conformer model can be used to identify a binding compound that is specific for polypeptides of a pharmacofamily. For example, the conformer model can be
5 compared to a structure of a compound or to a bound conformation of a ligand to identify those having similar conformation. A conformer model can be further used to query a database of compounds to identify individual compounds having similar conformations.

10 A conformer model of the invention can also be used to design a binding compound that is specific for polypeptides of one or more pharmacofamilies. The methods of the invention provide a conformer model that can be produced according to a cluster of bound
15 conformations of a ligand that are specific for polypeptides of a pharmacofamily. A conformer model identified by these criteria can be used as a scaffold structure for developing a compound having enhanced binding affinity or specificity for polypeptides of a pharmacofamily. Such a scaffold can also be used to
20 design a combinatorial synthesis producing a library of compounds which can be screened for enhanced binding affinity for polypeptide members of a pharmacofamily or specificity for polypeptide members of one pharmacofamily
25 compared to polypeptide members of another pharmacofamily. An algorithm can be used to design a binding compound based on a conformer model including, for example, LUDI as described by Bohm, J. Comput. Aided Mol. Des. 6:61-78 (1992).

30 A conformer model need not include all atoms of a pharmacocluster. Thus, a conformer model can include a

portion of atoms in a pharmacocluster so long as the portion consists of contiguous atoms of a bound conformation of a ligand and provides sufficient information to distinguish one pharmacocluster from
5 another. Thus, a conformer model can be constructed by overlaying corresponding fragments of bound conformations of a ligand and obtaining an average structure according to the methods described above. A conformer model made from a portion of a ligand can be advantageous due to its
10 small size compared to a complete structure of the ligand from which it was derived. A conformer model based on a portion of a bound conformation of a ligand can also be used to more efficiently and rapidly query a database due to a reduced use of computer memory compared to the
15 memory required to manipulate and store a structure containing all atoms of the ligand.

The invention provides a ligand conformer model, selected from the group consisting of conformer model 1 having coordinates listed in Table 3C, conformer
20 model 2 having coordinates listed in Table 4C, conformer model 3 having coordinates listed in Table 5C, conformer model 4 having coordinates listed in Table 6C, conformer model 5 having coordinates listed in Table 7C, conformer model 6 having coordinates listed in Table 8C, conformer
25 model 7 having coordinates listed in Table 9C, and conformer model 8 having coordinates listed in Table 10C. Conformer models 1-8 are average structures calculated from pharmacoclusters 1-8 respectively. The conformer models were determined as described in Example III and
30 are shown in Figure 4.

The invention also provides moiety, having coordinates listed in Table 3C, coordinates listed in Table 4C, coordinates listed in Table 5C, coordinates listed in Table 6C, coordinates listed in Table 7C, 5 coordinates listed in Table 8C, coordinates listed in Table 9C, or coordinates listed in Table 10C or subsets of the respective coordinate sets thereof. In one embodiment the moiety is not nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide 10 phosphate.

Additionally, the invention provides a method for constructing a pharmacophore model by constructing a model that contains one or more selected conformation-dependent properties of one or more pharmacoclusters. A 15 method for constructing a pharmacophore model can include the steps of (a) determining bound conformations of a ligand bound to different polypeptides; (b) identifying two or more bound conformations of the ligand having substantially the same bound conformation; (c) 20 identifying a conformation-dependent property of the bound conformations of the ligand having substantially the same bound conformation, the conformation-dependent property being correlated with the bound conformation of the ligand, and (d) constructing a model that contains 25 one or more selected conformation-dependent properties of one or more pharmacoclusters.

Additionally, a method for constructing a pharmacophore model can include the steps of (a) determining bound conformations of a ligand, or portion 30 thereof, bound to different polypeptides; (b) clustering two or more bound conformations of the ligand, or portion

thereof, having substantially the same bound conformation, thereby identifying a pharmacocluster, and (c) determining an average structure of the bound conformations of the ligand, or portion thereof, in the 5 pharmacocluster, wherein the average structure is a pharmacophore model. A method for constructing a ligand conformer model can also include the steps of (a) determining a bound conformation of a ligand, or portion thereof, bound to a polypeptide; (b) determining a 10 pharmacocluster having substantially the same bound conformation as the bound conformation, thereby identifying the bound conformation of the ligand as a member of the pharmacocluster, and (c) determining an average structure of the bound conformations of the 15 ligand in the pharmacocluster, wherein the average structure is a pharmacophore model.

A pharmacophore model constructed by the methods of the invention can be derived from any conformation-dependent property that is correlated with a 20 pharmacocluster. An example of a pharmacophore model useful in the methods of the invention is a conformer model. Additionally, a pharmacophore model can include a portion of a bound conformation, wherein the portion need not contain contiguous atoms of a bound conformation of a 25 ligand so long as the pharmacophore model provides sufficient information to distinguish one pharmacocluster from another. Thus, a pharmacophore model can appear as points in space unconnected by any semblance of a covalent bond due to absence of intervening atoms. For 30 example, a pharmacophore model constructed from a pharmacocluster of nicotinamide adenine dinucleotide bound conformations can contain a phosphate moiety and

nicotinamide ring moiety absent the ribose moiety which intervenes in a complete model of the structure.

A pharmacophore model can be any representation of points in a defined coordinate system that correspond 5 to positions of atoms in a bound conformation of a ligand. For example, a point in a pharmacophore model can correlate with the center of an atom in a conformer model. An atom of a conformer model can also be represented by a series of points forming a line, plane 10 or sphere. A line, plane or sphere can form a geometric representation designating, for example, shape of one or more atoms or volume occupied by one or more atoms.

A pharmacophore model can be represented in any coordinate system including, for example, a 2 dimensional 15 Cartesian coordinate system or 3 dimensional Cartesian coordinate system. Other coordinate systems that can be used include a fractional coordinate system or reciprocal space such as those used in crystallographic calculations which are described in Stout and Jensen, *supra*.

20 In addition to a geometric description of a bound conformation of a ligand, a pharmacophore model can include other characteristics of atoms or moieties of the ligand including, for example, charge or hydrophobicity. Thus, a pharmacophore model can be a generalized 25 structure, which includes but does not unambiguously describe the bound conformations of the ligand bound to the polypeptides in the pharmacofamily from which it was derived. For example, atoms can be represented as units of charge such that an oxygen in a bound conformation of 30 a ligand can be represented by an electronegative point

in the pharmacophore model. In this example, the electronegative point in the pharmacophore model includes any electronegative atom at that particular location including, for example, an oxygen or sulfur.

5 A pharmacophore model can be constructed to include, in addition to characteristics of the ligand itself, characteristics of an atom or moiety that interacts with the ligand and from a bound polypeptide. Characteristics of an interacting polypeptide atom or
10 moiety that can be included in a pharmacophore model include, for example, atomic number, volume occupied, distance from an atom of the ligand, charge, hydrophobicity, polarity, or location relative to the ligand. Methods for constructing a pharmacophore model
15 to include interacting atoms from a polypeptide are provided in Example III.

A characteristic included in a pharmacophore model can be incorporated into a geometric representation using any additional representation that can be
20 correlated with the characteristic. For example, use of color or shading can be used to identify regions having characteristics such as charge, polarity, or hydrophobicity. As such, the depth of shading or color or the hue of color can be used to determine the degree
25 of a characteristic. By way of example, a common convention used in the art is to identify regions of increased positive charge with deeper shades of blue, areas of increased negative charge with deeper shades of red and neutral regions with white. Numeric
30 representations can also be used in a pharmacophore model

including, for example, values corresponding to potential energy for an interaction, or degree of polarity.

In addition, a pharmacophore model can incorporate constraints of a physical or chemical property of the bound conformations of a ligand in a pharmacocluster. A constraint of a physical property can be, for example, a distance between two atoms, allowed torsion angle of a bond, or volume of space occupied by an atom or moiety. A constraint of a chemical property can be, for example, polarity, van der Waals interaction, hydrogen bond, ionic bond, or hydrophobic interaction. Such constraints can be included in a pharmacophore model using the representations described above.

A pharmacophore model can include two or more pharmacoclusters. In order to identify a ligand having broad specificity for two or more polypeptide pharmacofamilies, a pharmacophore model can be derived from the two or more corresponding pharmacoclusters. Additionally, in order to identify a ligand that can preferentially bind a first polypeptide which belongs to a first polypeptide pharmacofamily compared to a second polypeptide of a second polypeptide pharmacofamily, a pharmacophore model can incorporate constraints on geometry or any other characteristic so as to exclude a characteristic of the bound conformation of the ligand bound to the second polypeptide. For example, a geometric constraint can be a forbidden region for one or more atom of a bound conformation of a ligand. A forbidden region can be identified by overlaying two conformer models in a coordinate system and identifying a coordinate or set of coordinates differentially occupied

by one or more atoms of the conformer models. A pharmacophore model incorporating a forbidden region as such will be specific for a polypeptide of one pharmacofamily over a polypeptide of a second 5 pharmacofamily correspondent with the constraint incorporated.

An advantage of the invention is that a pharmacophore model can be created based on multiple 10 structures of the same ligand. In comparison to a pharmacophore model derived from a single structure or different ligands, a pharmacophore model derived from multiple bound conformations of the same ligand can include a greater degree of geometric information. For 15 example, averaging of multiple bound conformations of the same ligand can provide torsion angle constraints that are not available from a single structure and not evident from comparing different ligands.

20 The invention further provides a method for identifying a binding compound for one or more members of a polypeptide pharmacofamily by identifying a compound having a selected conformation-dependent property of a pharmacocluster. A binding compound can be any molecule 25 having selected conformation-dependent properties of a ligand such that the binding compound can form a complex with one or more members of one or more polypeptide pharmacofamily. A method for identifying a binding compound for one or more members of a polypeptide 30 pharmacofamily can include the steps of contacting a ligand with a polypeptide member of a pharmacofamily; identifying a conformation-dependent property associated with a bound conformation of the ligand bound to the

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polypeptide; comparing the conformation-dependent property of the bound conformation of the ligand bound to the polypeptide with a conformation-dependent property of a bound conformation of a ligand bound to another 5 polypeptide in the same pharmacofamily; and identifying a ligand bound to the polypeptide with a conformation-dependent property similar to a bound conformation of a ligand bound to another polypeptide in the same pharmacofamily, thereby identifying a compound that binds 10 one or more polypeptide members of a pharmacofamily. A compound that binds to one or more members of a polypeptide pharmacofamily can be identified by determining a conformation-dependent property by any of the methods described herein. For example, a ligand 15 conformation or spectroscopic signal can provide a conformation-dependent property useful in identifying a compound that binds to one or more members of a polypeptide pharmacofamily.

The methods described herein for identifying a 20 binding compound for one or more members of a polypeptide pharmacofamily can readily be adapted to a high throughput screening method. For example, methods of rapidly detecting a conformation-dependent property in a sequence of samples or detecting a conformation-dependent 25 property in parallel samples can be applied to a high-throughput screen. One skilled in the art will know how to adapt the methods described here to a high throughput screening format using, for example, robotic manipulation of samples.

30 A method for identifying a binding compound for one or more members of a polypeptide pharmacofamily can

include the steps of determining a bound conformation of a ligand bound to a polypeptide member of a polypeptide pharmacofamily; comparing the bound conformation of the ligand bound to the polypeptide member of the polypeptide 5 pharmacofamily to a pharmacophore model; and identifying the bound conformation of the ligand bound to the polypeptide member of the polypeptide pharmacofamily that satisfies the constraints of the pharmacophore model as a binding compound for one or more members of the 10 pharmacofamily in which the polypeptide member belongs.

A pharmacophore model can be useful in querying a database of polypeptide structures to find other members of a polypeptide pharmacofamily. For example, a member of a polypeptide pharmacofamily can be identified 15 by querying a database of bound conformations of a ligand to retrieve a structure that fits the constraints of the query pharmacophore model, thereby identifying the retrieved polypeptide as a member of the pharmacofamily from which the pharmacophore model was derived. A 20 pharmacophore model can also be used to identify a new member of a polypeptide pharmacofamily by querying a database of one or more polypeptide structures using an algorithm that docks or compares the pharmacophore model to polypeptide structures, wherein a favorable docking or 25 comparison identifies a polypeptide as a member of the same polypeptide pharmacofamily from which the pharmacophore model was derived. The database queries described above can be performed with algorithms available in the art including, for example, THREEDOM and 30 CATALYST.

An advantage of the invention is that a pharmacophore model can also be used to identify a binding compound that is specific for polypeptides of one or more pharmacofamilies. For example, a pharmacophore 5 model can be compared to a structure of a compound or to a bound conformation of a ligand to identify those having similar properties. A conformer model can be further used to query a database of compounds to identify individual compounds having similar properties.

10 A pharmacophore model of the invention can also be used to design a binding compound that is specific for polypeptides of one or more pharmacofamilies. A pharmacophore model identified by these criteria can be used as a scaffold or set of constraints for developing a 15 compound having enhanced binding affinity or specificity for polypeptides of one or more pharmacofamilies. Using similar methods a pharmacophore model can be used to design a combinatorial synthesis producing a library of compounds having properties consistent or similar to the 20 model which can be then be screened for enhanced binding affinity or specificity for polypeptide members of one or more pharmacofamilies. An algorithm can be used to design a binding compound based on a pharmacophore model including, for example, LUDI as described by Bohm, J. 25 Comput. Aided Mol. Des. 6:61-78 (1992).

A compound can be identified as satisfying the constraints of a pharmacophore model by a variety of methods for comparing structures. For example, a pharmacophore model that is a geometric representation 30 such as a conformer model can be overlaid with a compound, and the best fit determined as described

herein. Substantial overlap between a compound and a pharmacophore model can be indicated by a visual comparison and/or computation based comparison based on for example, RMSD values or torsion angle values as 5 described above. In a case where a pharmacophore model is represented by constraints, a compound can be fitted to the pharmacophore model to identify if the properties of the compound satisfy the constraints of the pharmacophore model. For example, if a pharmacophore 10 model contains, as a constraint, a maximum distance between atoms, a compound that satisfies the constraint can be identified as having a bond distance between corresponding atoms that is at least the maximum value. One skilled in the art will know how to extend such 15 methods of comparison to any physical or chemical constraint.

A compound can also be identified as satisfying the constraints of a pharmacophore model by demonstrating the same characteristics for one or more specific atom 20 located within a volume of space defined by the geometric constraints of the pharmacophore model. For example, in a case where polarity is a constraint and where a conformation of a compound can be overlaid with a pharmacophore model, an atom that overlaps a volume of 25 space indicated by the pharmacophore and having polarity within the defined limits can be identified as satisfying constraints of the pharmacophore. By extension, a compound having atoms which satisfy all constraints of a pharmacophore is identified as a binding compound for one 30 or more members of a polypeptide pharmacofamily from which the pharmacophore was produced.

Therefore, the invention provides a binding compound identified by the above described methods. For example, the invention provides a binding compound identified using a pharmacophore model or a conformer model derived from a pharmacocluster and/or pharmacofamily.

The invention provides a pharmacophore model, selected from the group consisting of pharmacophore model 1 having coordinates listed in Tables 3B and 3C, pharmacophore model 2 having coordinates listed in Tables 4B and 4C, pharmacophore model 3 having coordinates listed in Tables 5B and 5C, pharmacophore model 4 having coordinates listed in Tables 6B and 6C, pharmacophore model 5 having coordinates listed in Tables 7B and 7C, pharmacophore model 6 having coordinates listed in Tables 8B and 8C, pharmacophore model 7 having coordinates listed in Tables 9B and 9C, and pharmacophore model 8 having coordinates listed in Tables 10B and 10C.

The invention also provides a medium comprising a storage medium and stored in the medium, atom coordinates selected from the atomic coordinates listed in Table 3B, 3C, 4B, 4C, 5B, 5C, 6B, 6C, 7B, 7C, 8B, 8C, 9B, 9C, 10B or 10C, or a subset thereof. In one embodiment the medium comprises a computer readable medium. The use of a computer apparatus is convenient since atomic coordinates can be conveniently stored and accessed for manipulation including, for example, docking to a polypeptide structure or comparison to coordinates for other bound conformations of a ligand. Exemplary methods for manipulating atomic coordinates are described above.

It is understood that a computer apparatus of the invention need not itself store atomic coordinates of the invention. The computer apparatus contains an algorithm for viewing a structure from the coordinates or 5 otherwise manipulating the coordinates. By using various hardware, software and network combinations, the atomic coordinates can be manipulated in a variety of configurations. Such a separate medium can be another computer apparatus, a storage medium such as a floppy 10 disk, Zip disk or a server such as a file-server, which can be accessed by a carrier wave such as an electromagnetic carrier wave. One skilled in the art will know or can readily determine appropriate hardware, software or network interfaces that allow interconnection 15 of an invention computer apparatus.

The methods of the invention described herein can be performed in a computer apparatus using the atomic coordinates listed in Table 3B, 3C, 4B, 4C, 5B, 5C, 6B, 6C, 7B, 7C, 8B, 8C, 9B, 9C, 10B or 10C by adding the step 20 of entering the coordinates or a subset of the coordinates to the computer apparatus that performs a method of the invention. One skilled in the art will know or can readily determine an algorithm instructing a computer apparatus to carry out the methods of the 25 invention.

The invention provides a method for identifying a polypeptide that binds a ligand. The method includes the steps of (a) comparing a sequence of a polypeptide to a sequence model for polypeptides that bind a ligand; and 30 (b) determining a relationship between the sequence and the sequence model, wherein a correspondence between the

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sequence and the sequence model identifies the polypeptide as a polypeptide that binds the ligand.

A method for identifying a polypeptide that binds a ligand can include the steps of (a) comparing a 5 sequence of a polypeptide to a sequence model for polypeptides that bind a ligand, wherein the sequence model comprises representations of amino acids consisting of a subset of amino acids, the subset of amino acids having one or more atom within a selected distance from a 10 bound ligand in the polypeptides that bind the ligand; and (b) determining a relationship between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence model identifies the polypeptide as a polypeptide that binds the ligand.

15 The invention also provides a method for identifying a member of a pharmacofamily. The method includes the steps of (a) comparing a sequence of a polypeptide to a sequence model for polypeptides of a pharmacofamily; and (b) determining a relationship 20 between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence model identifies the polypeptide as a member of the pharmacofamily.

25 According to the methods of the invention, a sequence can be identified as being similar to polypeptides in a set of polypeptides. A polypeptide set can be represented by a sequence model identifying similarity between the sequences of the polypeptides in 30 the set. A sequence model provides a mathematical representation of a linear sequence of symbols including,

for example, symbols representing amino acids or gaps in a polypeptide sequence. A sequence model provides relative probabilities for each amino acid type occurring at each position in a polypeptide sequence. Model

5 parameters can be set based on the frequency of amino acids at each position in a set of polypeptide sequences or other factors including, for example, naturally occurring distributions such as with Dirichlet mixture in a Hidden Markov Model as described in Durbin et al.,
10 *supra*. Thus, a sequence model can provide a statistical model to which new sequences can be compared to determine if the new sequence is similar to polypeptides in the set from which the model was generated.

Sequence models and methods for making and using sequence models are well known in the art as described for example in Durbin et al., *supra*. Several types of sequence models can be used in the methods of the invention including, for example, Hidden Markov Models (HMM) which have been described, for example, in Eddy, Bioinformatics 14:775-63 (1998), Position Specific Score Matrices (PSSM) which have been described, for example, in Gribskov et al., Proc. Natl. Acad. Sci. USA, 84:4355-58 (1987), Support Vector Machines (SVM) which have been described, for example, in Jaakkola et al., J. Computational Biology 7:95-114 (1999), or Neural Networks as described, for example, in Baldi and Brunak Bioinformatics: The Machine Learning Approach MIT Press, Cambridge, MA (1998).

A sequence model can be produced from a variety
30 of polypeptide sets containing polypeptides with similar
sequences. A polypeptide set used to produce a sequence

model can be referred to as a training set and the resultant sequence model can be referred to as trained by the polypeptide set. A sequence model provides a statistical description of the occurrence of specific 5 amino acids at specified positions in a training set of polypeptides. An advantage of a sequence model is that it can be produced in cases where an alignment has not been produced or to identify similarities not evident in a traditional pairwise alignment such as BLAST (Altschul 10 et al., J. Mol. Biol. 215:403-410 (1990)) or FASTA (Pearson and Lipman, Proc Natl. Acad. Sci. USA 85:2444-2448 (1998)).

A sequence model can be produced using full sequences of polypeptides or portions of a polypeptide 15 sequence. A portion of a polypeptide useful in making a sequence model of the invention can include, for example, a region of sequence identified by structural criteria such as correlation with a domain or polypeptide fold or functional criteria such as correlation with a binding 20 activity, enzymatic activity or other biological activity. A portion of a polypeptide useful in producing a sequence model can also include positions of amino acids that are not contiguous in the polypeptide from which they are derived. For example, a subset of amino 25 acids can be identified according to structural criteria such as proximity in the three dimensional structure or functional criteria such as participation in a binding activity, enzymatic activity or other biological activity of a polypeptide.

30 Therefore, a sequence model of the invention can contain representations of amino acids consisting of

a subset of amino acids, the subset of amino acids having one or more atom within a selected distance from a bound ligand in a set of polypeptides. A sequence model of the invention can be produced by the steps of: (a) 5 identifying a subset of amino acids having one or more atom within a selected distance from a bound conformation of a ligand in a set of polypeptides that bind the ligand; and (b) producing a sequence model, amino acids of the sequence model consisting of the subset of amino 10 acids.

In addition, a sequence model of the invention can contain representations of amino acids consisting of a subset of amino acids, the subset of amino acids having one or more atom within a selected distance from a bound 15 ligand in the polypeptides of the pharmacofamily. A sequence model of the invention can be produced by the steps of: (a) identifying a subset of amino acids in a pharmacofamily having one or more atom within a selected distance from a bound conformation of a ligand; and (b) 20 producing a sequence model, amino acids of the sequence model consisting of the subset of amino acids. Exemplary methods for making a sequence model based on either full sequences of polypeptides in a set of polypeptides or based on a subset of positions in the sequences of 25 polypeptides in a set of polypeptides are provided in Examples VII, VIII and IX.

Comparison of a polypeptide sequence to sequences in a set of polypeptide sequences can be conveniently carried out by comparing the polypeptide 30 sequence to a sequence model produced from the polypeptide sequences in the set. Such a comparison can

indicate the likelihood that the sequence is accurately represented by the model, or that the sequence is a member of the set of polypeptides used to create the sequence model. A polypeptide with a high probability of 5 being similar to a sequence model can be identified as having a high probability of being a member of a set of polypeptides from which the sequence model was derived. For example, a sequence model can be produced based on the polypeptides in a pharmacofamily and this sequence 10 model can be used to search a database for new members of the respective pharmacofamily. Exemplary methods for producing a sequence model and using the model to identify new members of a pharmacofamily are described in Examples VII, VIII and IX.

15 A probability that a polypeptide sequence has a correspondence with a sequence model can be determined from a probability score. For example, HMMER, which is described in Examples VII to IX, can be used to compare one or more sequences to a Hidden Markov Model. HMMER 20 indicates the probability that a given sequence belongs to a pharmacofamily used to produce a Hidden Markov Model by reporting an E value for each sequence compared. Lower E values resulting from comparison of a sequence to a sequence model correspond to a stronger probability 25 that the compared sequence belongs to a pharmacofamily used to produce the sequence model. Therefore, an E value can be used to determine whether a similarity between a sequence and sequence model is statistically relevant.

30 A statistically relevant similarity can be identified as having an E value less than a desired

cutoff value. An E value below 1 can be considered to indicate a correspondence, or a high probability of correspondence. Increasing the E value cutoff will include a larger number of sequences as corresponding to 5 the sequence model. Thus, a larger E value cutoff can be used in cases where it is desired to minimize the number of members of the pharmacofamily that are missed. More specifically, increasing the E value will increase the percentage of true positives identified. Increasing the 10 number of true positives identified can be achieved by increasing the E value cutoff, for example, to 2, 5, 10, 50 or 100 or higher. An increased E value will also increase the percentage of false positives identified. In cases where it is desired to minimize incorrectly 15 identified sequences, the E value cutoff can be decreased, for example, to 0.5, 0.2, 0.1 or 0.01 or lower. Thus, one skilled in the art can determine an appropriate E value based on the desired or tolerable numbers of true and false positives identified.

20 An E value cutoff can also be made according to the shape of a curve in a plot of $-\ln(E)$ versus L, where L is the location of compared sequences in a list ranked by descending E value. For example, an E value cutoff can be identified as a significant inflection in the 25 curve. An inflection point is that point where the second derivative of $-\ln(E)$ with respect to L is zero. An inflection in the curve that identifies an appropriate E value cutoff can be identified by its magnitude and/or position relative to a specified E value. For example, 30 an E value cutoff for determining statistically relevant similarity can be at a statistically significant inflection point before a specified threshold value of E

is reached in a plot of $-\ln(E)$ versus L, or at the last inflection point before a specified threshold value of E in such a plot. A statistically significant inflection point can be identified as having a $-\ln(E)$ before the
5 inflection point that differs from $-\ln(E)$ after the inflection point by at least 50. Smaller differences in $-\ln(E)$ at the inflection point including, for example, at least 10, at least 5, at least 2, at least 1.5 or at least 1 or lower can identify a cutoff for statistically
10 relevant similarity, for example, when longer sequence subsets are used or when sequence models are compared to relatively long sequences. In addition, a cutoff for statistically relevant similarity can be indicated by a larger difference in $-\ln(E)$ value at the inflection
15 including, for example 100, or 500 or higher, for example, when shorter sequence subsets are used or when sequence models are compared to relatively short sequences. Examples of determining E value cutoffs according to the shape of a plot of $-\ln(E)$ versus L are
20 provided in Examples VII and VIII.

A member of a pharmacofamily can also be identified by determining relative E values from the set of E values determined for sequences identified in a search of a database using a sequence model. As
25 demonstrated in Example X, a relative E value can be a cross correlation value (XCorr) which is calculated as follows: an E value is determined for a particular sequence based on a search of a database using a sequence model, the natural log of this E value is calculated ($-\ln(E)$), and XCorr is calculated as the ratio of the $-\ln(E)$ for the particular sequence to the summed $-\ln(E)$ for all pharmacofamilies. Differences in XCorr values for

candidate sequences identified in a sequence search can be used to identify members that are included and excluded from a particular pharmacofamily. As demonstrated in Example IX, a plot of XCorr values vs. L 5 can be particularly useful in identifying members of a pharmacofamily in cases where the magnitude of the drop position between members and nonmembers in a plot of - ln(E) vs. L is relatively small.

In general, sequence members of a 10 pharmacofamily can be identified as having an XCorr value larger than about 0.5. XCorr values larger than 0.5 such as 0.6, 0.7, 0.8, 0.9 or 1 indicate that the probability that the sequence belongs to the specified pharmacofamily is much higher than the probability that it belongs to a 15 different pharmacofamily. Sequences with an XCorr value close to zero for a given pharmacofamily have a greater probability of belonging to another pharmacofamily.

The methods of parsing protein sequences into 20 pharmacofamilies described herein are useful for identifying structurally related proteins such as 25 proteins having structurally related binding sites. The methods for identifying pharmacofamilies and members thereof can be used in combination with gene family based drug discovery methods, such as those described in WO- 09960404 (1999, Triad Therapeutics Inc (Sem DS): Multi-partite ligands and methods of identifying and using same), to find inhibitors having nanomolar affinity for 30 members of one or more pharmacofamily. Using such methods focused chemical libraries of potential inhibitors can be designed and synthesized, or otherwise identified and obtained based on the common structural

properties of the binding sites of protein members of a particular pharmacofamily. These focused libraries can be screened to identify inhibitors having high affinity for members of a particular pharmacofamily. The
5 inhibitors can be further screened for specificity toward members of one pharmacofamily compared to members of other pharmacofamilies within the same gene family. Thus, methods of assigning a protein to a pharmacofamily based on amino acid sequence alone, such as those
10 described in Example X and employed by the Gene Family Profiler program described therein, can increase the efficiency at which high affinity inhibitors are identified.

One skilled in the art will be able to identify
15 a statistically relevant similarity between an identified sequence and a sequence model based on any known method of statistical analysis including, for example, those that use scores other than E values. Based on the description herein, which has been exemplified with E
20 scores, one skilled in the art will be able to adapt a variety of statistical analysis methods to the methods of the invention.

The methods of the invention can be performed in an iterative fashion where E value cut offs are
25 adjusted until a desired set of sequences are identified. A desired set can be, for example, a validation set as described in Examples VII and VIII. A validation set is understood to be a collection of polypeptides including all known members of a group of polypeptides such as a
30 pharmacofamily.

Iterations in the methods of the invention can also include modifying the training set based on newly identified members of a set of polypeptides to improve the sequence model. Thus, the methods of the invention

5 can include the steps of (a) comparing a sequence of a polypeptide to a sequence model for polypeptides that bind a ligand; (b) determining a relationship between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence model identifies

10 the polypeptide as a polypeptide that binds the ligand; (c) producing a sequence model with a set of sequences, the set of sequences consisting of sequences of polypeptides having a subset of amino acids, the subset of amino acids having one or more atom within a selected

15 distance from a bound ligand in said polypeptides that bind said ligand; (d) adding the sequence of the identified polypeptide that binds the ligand to the set of sequences; and (e) repeating steps (a) through (c) one or more times. In addition steps (a) through (d) can be

20 repeated multiply to iteratively improve the sequence model. For example, the method can be repeated 2 or more times, 3 or more times, 5 or more times, or 10 or more times.

The method can also be iterated according to

25 the following steps (a) comparing a sequence of a polypeptide to a sequence model for polypeptides of a pharmacofamily; (b) determining a relationship between the sequence and the sequence model, wherein a correspondence between the sequence and the sequence

30 model identifies the polypeptide as a member of the pharmacofamily; (c) producing a sequence model with a set of sequences, the set of sequences consisting of

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sequences of polypeptides in the pharmacofamily; (d) adding a sequence of the identified member of the pharmacofamily to the set of sequences; and (e) repeating steps (a) through (c) one or more times.

5 An ideal sequence comparison method would find all true positives and no false positives. In practice, a trade-off between these two goals is often required. A search can be either sensitive enough to find all true positives, but find false positives as well, or selective
10 enough to find no false positives, but then miss some of the true positives. The method of differential filtering can be used to minimize this trade-off as described below.

15 The invention also provides a method for identifying a member of a pharmacofamily, wherein the method includes the steps of (a) comparing a sequence of a polypeptide to a sequence model and a differential sequence model; and (b) determining a relationship
20 between the sequence and the sequence models, wherein a correspondence between the sequence and the sequence models identifies the polypeptide as a member of the pharmacofamily. The method can further include the following steps: (c) producing a sequence model with a
25 set of sequences, the set of sequences consisting of sequences of polypeptides in the pharmacofamily; (d) adding a sequence of the identified member of the pharmacofamily to the set of sequences; and (e) repeating steps (a) through (c) one or more times. In addition
30 steps (a) through (d) can be repeated multiply to iteratively improve the sequence model. For example, the

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method can be repeated 2 or more times, 3 or more times, 5 or more times, or 10 or more times.

The discriminative ability of a sequence model to identify members of a set of polypeptides can be 5 augmented by creating multiple models having differential discriminative modes. Differential sequence models can represent, or emphasize, different aspects of a set of polypeptides. For example, a first model representing a structural alignment of polypeptides in a pharmacofamily 10 can represent different aspects of the pharmacofamily members than a second, differential model emphasizing a binding site region of the same polypeptides.

Sequentially filtering the identified sequences from one sequence model with a second differential sequence model 15 screen reduces the rate of false positives overall. This is demonstrated in Example VII where it is shown that differential filtering can provide a decrease in the number of falsely identified sequences while minimizing the decrease in the percentage of correctly identified 20 sequences.

Different types of sequence models can be used to compare sequences by differential filtering. For example, the identified sequences from a database search with a Hidden Markov model can be sequentially filtered 25 with a Neural Network model. Furthermore, differential filtering can be performed with a combination of different amino acid training sets and different types of sequence models. For example, the identified sequences from a database search with a Hidden Markov model trained 30 with all of the amino acid positions present in a structural model of a polypeptide can be filtered with a

Neural Network model trained with a subset of amino acid positions including those residues that are proximal to a bound ligand. Although the above examples describe differential filtering in a sequential mode, it is
5 understood that differential sequence models can also be compared to one or more sequence in a parallel mode and the results compared to identify sequences similar to polypeptides in a set such as a pharmacofamily.

A determination as to whether differential
10 filtering should be used can be made from the shape of a plot of $-\ln(E)$ versus L produced as described above. If there is a sharp drop in E value, a large second derivative, and all the known members among the identified sequences occur at lower E value compared to
15 the location of the drop, then one model can be adequate. However, if the curve does not have significant inflections or known members occur at higher scores than a significant inflection, then a clear E value cutoff can be difficult to determine. In such cases, choosing a
20 liberal E value cutoff, sufficient to include all true positives, and applying differential filtering to the resulting subset of sequences, can be used to decrease the number of false positives while minimizing a decrease in the number of true positives.

When multiple sequence models are used, it can be advantageous to increase the E value cutoff for sequence models based on short sequences or small amino acid position sets, as shorter sequences tend to produce larger E values. An appropriate cutoff to use can be
30 determined from test runs on a validation set of known

matches and mismatches, such as described in Examples VII and VIII.

Validation of a sequence model can also be accomplished using only part of the known members of a pharmacofamily to produce, or train, a sequence model and the ability of the model to find members in a database can be tested. In such a case the members in the database that were left out of the training set will be scored lower (higher E value) than those included in the training set. The score of the omitted sequences can indicate a relative upper limit (smallest E value) of an appropriate cutoff when a model trained with all known members is used to search for new and/or unknown members. A sequence which scores in the same region as the omitted known members has a significant probability of being a member whatever the E value.

The methods of the invention can also be used to distinguish to which set of polypeptides an identified polypeptide belongs. For example, the methods can be used to determine which pharmacofamily a polypeptide belongs. As described above a number of pharmacofamilies can be identified within a family of polypeptides. A sequence of a polypeptide member of a family can be compared to sequence models derived from each pharmacofamily within the family of polypeptides. Based on probability scores for the relationship of the polypeptide sequence to each sequence model, the pharmacofamilies to which the sequence is most likely to belong can be determined. Specifically, the sequence would have the highest probability of belonging to the

pharmacofamily used to derive the sequence model for which the most favorable probability score resulted.

The probability that a sequence belongs to, or is accurately modeled by, a particular sequence model can 5 easily be determined, for example, by comparison of probability scores such as E values. A matrix of probability scores for all known members of a polypeptide family with each pharmacofamily sequence model can be used to expose any gaps in the coverage of the family by 10 the pharmacofamily sequence models. The gaps can be correlated to outlying sequences that were not adequately modeled by any of the pharmacofamily sequence models. The number of such gaps indicates the degree to which the collection of pharmacofamily sequence models form a basis 15 set that spans the sequence space of the polypeptide family.

Based on the conformations of a ligand identified from pharmacoclusters associated with each pharmacofamily a binding compound can be identified or 20 designed as described herein previously. Thus, a polypeptide sequence can be identified and compared to a set of pharmacofamilies in a family of polypeptides to predict or determine specificity toward individual binding compounds based on conformation. Similar methods 25 of determining the probability that any sequence belongs to a pharmacofamily can be used to extend a pharmacofamily sequence model through a proteome such that members of a given pharmacofamily can be identified in the proteome, for example, as described in Example IX.

Although the above description has been made with reference to polypeptide sequences as examples, one skilled in the art will know that similar methods can be applied to sequence models derived from polynucleotide
5 sequences.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein.

10 Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

**Identification of Polypeptide Pharmacofamilies Based on
Bound Conformations of NAD(P)(H) Ligands**

15 This example describes identification of ligand conformer groups and corresponding polypeptide pharmacofamilies based on bound conformations of NAD(P)(H) bound to polypeptide oxidoreductases.

The oxidoreductases form a family of
20 polypeptides that bind NAD(H) and NADP(H). In order to identify pharmacofamilies within the family of oxidoreductases, bound conformations of NAD(P)(H) were determined by searching the protein databank. Bound conformations from 156 structures were clustered into
25 separate pharmacoclusters, and pharmacofamilies were identified according to binding to bound conformations of NAD(P)(H) in separate pharmacoclusters.

Structure files containing polypeptides with bound NAD(P) (H) were identified from the protein databank by keyword searches using the database software. Keywords included "NAD," "NADH," "NADP," "NADPH," 5 "oxidoreductase," "dehydrogenase" and "reductase." Cluster analysis was performed using the algorithm COMPARE (Chiron Corp, 1995; distributed by Quantum Chemistry program Exchange, Indianapolis IN) in combination with visual inspection. All clusters were 10 visually inspected using Insight 98 for outliers that demonstrated poor overlay with the rest of the pharmacocluster as a whole. These outliers were compared against each other and existing pharmacoclusters to find other possible matches. Those that did not fit any 15 family were removed. Comparison between bound conformations was made based on the RMSD equations supplied in COMPARE.

Eight pharmacoclusters were identified by this method, as shown in Figure 1. Visual inspection of the 20 clusters in Figure 1 demonstrates that members within a cluster are substantially overlapped. Comparison between clusters demonstrates substantial differences. For example, the bound conformations in cluster 5 have an extended structure compared to the bound conformations in 25 cluster 4, which form a horseshoe like shape. Other differences include, for example, a flip in the nicotinamide ring between cluster 1 and cluster 2 such that the nicotinamide ring is *anti* to the ribose in cluster 1 and *syn* to the ribose in cluster 2 and a change 30 in torsion angle in the bonds connecting the adenine ribose to the adenine phosphate for the bound

conformations of cluster 3 compared to those of cluster 2.

The dihedral angles for various bonds in the bound conformations of the NADP(H) ligand can be used to
5 distinguish the pharmacoclusters. As shown in Table 1 (see Figure 2 for atom and bond locations), although many dihedral angles are similar between two or more pharmacoclusters, each pharmacocluster can be distinguished from the others by comparison of the full
10 set of dihedral angles. For example, pharmacoclusters 2 and 3 can be distinguished by comparison between the dihedral angles at O4'A-C4'A-C5'A-O5'A which are 154 degrees and -131 degrees respectively and by comparison between the dihedral angles at C5'A-O5'A-PA-O3 which are
15 105 degrees and 57 degrees respectively.

Table 1: Dihedral Angles for Pharmacoclusters

| Dihedral angle | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 |
|-------------------------|------|-----|------|-----|------|-----|------|-----|
| | Avg. | std | Avg. | std | Avg. | std | Avg. | std |
| O4 'A-C1 'A- N9A-C8A | 75 | 24 | 75 | 11 | 69 | 18 | 85 | 7 |
| O4 'A-C4 'A-C5 'A-O5 'A | 180 | 19 | 154 | 30 | -131 | 99 | -166 | 12 |
| C4 'A-C5 'A-O5 'A- PA | 138 | 86 | 137 | 15 | 121 | 93 | -152 | 2 |
| C5 'A-O5 'A- PA- | 65 | 39 | 105 | 44 | 57 | 44 | 55 | 0 |
| O5 'A- PA- O3 - | 97 | 61 | 42 | 77 | 74 | 24 | 115 | 20 |
| PA- O3 - PN-O5 'N | -143 | 72 | -165 | 53 | -136 | 29 | -152 | 10 |
| O3 - PN-O5 'N-C5 'N | 70 | 44 | 56 | 86 | 101 | 36 | -64 | 22 |
| PN-O5 'N-C5 'N-C4 'N | 181 | 14 | 176 | 41 | 162 | 27 | 145 | 7 |
| O5 'N-C5 'N-C4 'N-O4 'N | -73 | 46 | -58 | 40 | -54 | 26 | -55 | 10 |
| O4 'N-C1 'N- N1N- C2 N | -120 | 24 | 69 | 17 | 53 | 11 | 59 | 5 |
| C1 'A-C2 'A-C3 'A-C4 'A | -25 | 10 | -2.9 | 5 | -29 | 10 | -37 | 23 |
| C1 'N-C2 'N-C3 'N-C4 'N | -36 | 44 | -35 | 6 | -28 | 20 | 22 | 9 |

A quantitative analysis of the results of clustering bound conformations of NAD(P)(H) is provided in Table 2. Table 2 shows RMSD values calculated from comparisons between each pharmacocluster's average coordinates. Average coordinates were determined from the pharmacocluster subsets listed in Tables 3 through 10 as described below.

Tables 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10A show RMSD values for subsets of members of pharmacoclusters 1-8, respectively. The RMSD values for each member were calculated as comparisons to an average structure for the 5 subsets shown in each table respectively. For each pharmacocluster a subset of the possible ligands that belong to each cluster were identified. Each subset was chosen to maximize the diversity of the family and to minimize over-representation of ligand conformations from 10 enzymes that exist multiply in the PDB database. The goal of the subset selection was to fully represent characteristics from oxidoreductases belonging to a range of species and catalyzing a range of different reactions. For example, there exists over ten alcohol dehydrogenases 15 in the PDB database; however, for purposes of this study, only three were chosen from three different species for use in the 3D overlay and the pharmacophore construction. Average coordinates for the above described pharmacocluster subsets were obtained by overlaying 20 ligand structures in MSI InsightII using the overlay function. The three dimensional coordinates for each atom in each ligand were used to calculate an average position and a standard deviation for the pharmacofamily.

Comparison of the RMSD values in part A of 25 Tables 3 through 10 with the RMSD values in Table 2 demonstrate that a member of a pharmacocluster can be identified as having a lower RMSD compared to an average conformation of the members in its pharmacocluster than the RMSD between each family's average coordinates. In 30 some cases it can be beneficial to combine two or more methods of comparison. For example, as described above pharmacoclusters 2 and 3 which have a relatively low RMSD

when compared to each other can be distinguished from each other by visual inspection and by comparison of dihedral angles at various bonds.

- These results demonstrate that bound
5 conformations of a ligand can be grouped into pharmacoclusters by methods of structure comparison. These results also demonstrate methods for distinguishing pharmacoclusters and members within pharmacoclusters.

Example II

10. Correlation Between the Structure of Polypeptides and the Bound Conformations of NAD(P) (H)

This example describes a correlation between bound conformations of NAD(P) (H) and structural classification of polypeptides such that polypeptides of 15 a pharmacofamily have similar protein fold.

Pharmacoclusters for conformations of NAD(P) (H) bound to oxidoreductase polypeptides were clustered as described in Example I. For each polypeptide the protein fold, SCOP super-family designation and SCOP family 20 designation was identified from the SCOP website administered by Laboratory of Molecular Biology at the MRC, Cambridge England (<http://mrc-lmb.cam.ac.uk>).

Table 11 shows the grouping of NAD(P) (H) binding polypeptides into 8 pharmacofamilies.

Table 11: Pharmacofamilies

| Family 1: NAD (P) Rossman Binding Domain (anti) | | | | SCOP-Superfamily | SCOP-Family |
|--------------------------------------------------------|---------------|------------|------------------------|-------------------------|---------------------------|
| Polypeptide | Source | PDB | Fold | | |
| Alcohol Dehydrogenase | Horse Liver | 1a71 | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | human | 1agn | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | Human | 1dl1t | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | Horse Liver | 1axe | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | Horse Liver | 1axg | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | cod fish | 1cdo | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | Horse Liver | 1deh | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | Human | 1d1s | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | human | 1hdx | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |
| Alcohol Dehydrogenase | human | 1hdy | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydrog. |

| | | | | | |
|--------------------------------------|---------------------|------|------------------------|------------------------|-----------------------------|
| Alcohol Dehydrogenase | Horse Liver | 1hdz | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Horse Liver | 1hld | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | human | 1htb | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Cod liver | 1kev | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Horse Liver | 1lde | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | horse liver | 1ldy | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | human | 1teh | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Thermoan aerobium | 1ykf | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Horse Liver | 2ohx | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Horse Liver | 2oxi | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | Horse Liver | 3bt0 | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| Alcohol Dehydrogenase | human | 3hud | NAD(P) binding Rossman | NAD(P) binding Rossman | Alcohol/glucose dehydreg. |
| D-2-hydroxyisocaproate Dehydrogenase | Lactobacillus Casei | 1dxy | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydreg. |

| | | | | | |
|--------------------------------------|----------------------------|------|---------------------------|---------------------------|-------------------------------------------|
| D-3-Phosphoglycerate Dehydrogenase | E. Coli | 1psd | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrogenase. |
| Dihydriodipicolinate Reductase | E. Coli | 1arz | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehyde-3-phosphate dehydrogenase. |
| Dihydriodipicolinate Reductase | E. Coli | 1dih | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehyde-3-phosphate dehydrogenase. |
| Formate Dehydrogenase | Fyrobaculum Aerophilum | 1qp8 | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrogenase. |
| Formate Dehydrogenase | Methylotrophic Pseudomonas | 2nad | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrogenase. |
| L-2-hydroxyisocaproate dehydrogenase | Lactobacillus Confusus | 1hyh | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrogenase. |
| L-Alanine Dehydrogenase | Phormidium lapideum | 1pjc | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrogenase. |

| | | | | | |
|--------------------------------|---------------------------|------|---------------------------|---------------------------|-------------------------------------|
| L-Lactate Dehydrogenase | Plasmodium Falciparum | 11dg | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | Bacillus DelbreuKii | 11dl | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | B.Steari othermophilus | 11dn | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | Bifidobacterium Longum | 11ld | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | Bifidobacterium Longum | 11lh | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | B.Steari othermophilus | 21db | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |

| | | | | | |
|-------------------------|------------------------|------|------------------------|------------------------|-------------------------------------|
| L-Lactate Dehydrogenase | Pig Muscle | 91db | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| L-Lactate Dehydrogenase | Pig Muscle | 91dt | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | Aquaspirillum Arcticum | 1b8u | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | Thermus Flavis | 1bmd | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | E. coli | 1cme | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | E. coli | 1emd | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |

| Family 2 : NAD(P) Rossman Binding Domain (Syn) | SCOP-Superfamily | SCOP-Family | | | |
|------------------------------------------------|---------------------------|-------------|---------------------------|---------------------------|-------------------------------------|
| Polypeptide | Source | PDB | Fold | | |
| Malate Dehydrogenase | Haloarcula Marismortui | 1hlp | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | Pig Heart | 4mdh | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malate Dehydrogenase | Pig Heart | 5mdh | NAD(P) binding Rossman | NAD(P) binding Rossman | Lactate & malate dehydrog. (N-term) |
| Malic Enzyme | human | 1qr6 | NAD(P) binding Rossman | NAD(P) binding Rossman | Amino-acid dehydrog (C-term) |
| S-AdenosylHomocysteine Hydrolase | Rat | 1b3r | NAD(P) binding Rossman | NAD(P) binding Rossman | Formate/glycerate dehydrog. |
| Tetrahydrofolate Dehydrogenase | Human | 1a4i | NAD(P) binding Rossman | NAD(P) binding Rossman | Amino-acid dehydrog (C-term) |
| | | | | | |
| | | | | | |

| | | | | | |
|-------------------------------------------|------------------------|------|------------------------|------------------------|-------------------------------------------------|
| Glutamate Dehydrogenase | Bovine Liver | 1ch6 | NAD(P) binding Rossman | NAD(P) binding Rossman | Amino-acid dehydrog (C-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | Leishmania mexicana | 1a7k | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | Thermus aquaticus | 1cer | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | B. Stearo thermophilus | 1dbv | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | E. Coli | 1gad | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | E. Coli | 1gae | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |
| Glyceraldehyde -3-phosphate Dehydrogenase | B. Stearo thermophilus | 1gd1 | NAD(P) binding Rossman | NAD(P) binding Rossman | Glyceraldehydes-3-phosphate dehydrog . (N-term) |

| | | | | | |
|---------------------------------------------|---------------------------|------|-------------------------|-------------------------|----------------------------------------------------|
| Glyceraldehyde -3 - phosphate Dehydrogenase | Trypanosoma Brucei Brucei | 1ggg | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |
| Glyceraldehyde -3 - phosphate Dehydrogenase | Leishmania Mexicana | 1gyp | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |
| Glyceraldehyde -3 - phosphate Dehydrogenase | Thermatoga Marinata | 1hdg | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |
| Glyceraldehyde -3 - phosphate Dehydrogenase | Palinurus Versicolor | 1szj | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |
| Glyceraldehyde -3 - phosphate Dehydrogenase | B. Stearo thermophilus | 2dbv | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |
| Glyceraldehyde -3 - phosphate Dehydrogenase | B. Stearo thermophilus | 3dbv | NAD (P) binding Rossman | NAD (P) binding Rossman | Glyceraldehydes -3 - phosphate dehydrog . (N-term) |

| Family 3 : NAD(P) Rossman Binding Domain (Syn) | | | | | | Tyrosine Dependent Oxidoreductases | | |
|------------------------------------------------------|---------------------------|------|---------------------------|--|---------------------------|------------------------------------|--------------------|--|
| Polypeptide | Source | PDB | Fold | | | SCOP-Superfamily | SCOP-Family | |
| 17 β -Hydroxysteroid Dehydrogenase | Human | 1a27 | NAD(P) binding Rossman | | NAD(P) binding Rossman | | Tyrosine-dependent | |
| 2 α -20 β -Hydroxysteroid Dehydrogenase | Strep. Hydrogen ans | 2hsd | NAD(P) binding Rossman | | NAD(P) binding Rossman | | Tyrosine-dependent | |
| 7 α -Hydroxysteroid Dehydrogenase | E. Coli | 1ahh | NAD(P) binding Rossman | | NAD(P) binding Rossman | | Tyrosine-dependent | |
| 7 α -Hydroxysteroid Dehydrogenase | E. Coli | 1ahi | NAD(P) binding Rossman | | NAD(P) binding Rossman | | Tyrosine-dependent | |
| 7 α -Hydroxysteroid Dehydrogenase | E. Coli | 1fmc | NAD(P) binding Rossman | | NAD(P) binding Rossman | | Tyrosine-dependent | |

| Carbonyl Reductase | Mouse | 1cyd | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
|------------------------------------------------|---------------------------|------|----------------------------|----------------------------|------------------------|
| Cis-Biphenyl-2,3-Dihydrodiol-2,3-Dehydrogenase | Pseudomonas sp. | 1bdb | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Dihydropteridine Reductase | Rat Liver | 1dir | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Dihydropteridine Reductase | Human | 1hdr | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | M. Tuberculosis | 1bvr | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | Brassica Napus (rappe) | 1cwu | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | E. coli | 1dfg | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | E. coli | 1dfh | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | E. coli | 1dfi | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |
| Enoyl Acyl Carrier Protein Reductase | Myobacterium Tuberculosis | 1eny | NAD (P) binding Rossman | NAD (P) binding Rossman | Tyrosine- dependent |

| | | | | | |
|--------------------------------------|--------------------------|------|---------------------------|---------------------------|--------------------|
| Enoyl Acyl Carrier Protein Reductase | Mybacterium Tuberculosis | lenz | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Enoyl Acyl Carrier Protein Reductase | E. Coli | 1gg6 | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Enoyl Acyl Carrier Protein Reductase | Common Bacteria | 1qsg | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| GDP-Fucose Synthase | E. Coli | 1bsv | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Sepiapterin Reductase | E. Coli | 1nas | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Sepiapterin Reductase | mouse | 1sep | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Trihydroxynaphthalene Reductase | Rice Fungus | 1ybw | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Tropinone Reductase-I | Jimson Weed | 1ael | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| Tropinone Reductase-II | Jimsonweed | 2ae2 | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| UDP-Galactose Epimerase | E. Coli | 1a9Y | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| UDP-Galactose Epimerase | E. Coli | 1a9Z | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |
| UDP-Galactose Epimerase | E. Coli | 1kvq | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine-dependent |

| | | | | | |
|--------------------------------------------------------------|--------------------------|-------|---------------------------|---------------------------|------------------------|
| UDP-Galactose Epimerase | E. Coli | 1kvrl | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1kvs | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1kvvt | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1kvvu | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1nai | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1uda | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1udb | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1udc | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 1xel | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| UDP-Galactose Epimerase | E. Coli | 2hsd | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| 3 α , 20 β - hydroxysteroid dehydrogenase | Strep. Hydrogen as | | | | |
| 17- β hydroxy steroid Dehydr. | Human | 1fdu | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |
| 17- β hydroxy steroid Dehydr. | Human | 1fdv | NAD(P) binding Rossman | NAD(P) binding Rossman | Tyrosine- dependent |

| Family 4: Catalases | | | | Family 5: β - α TIM Barrel | | | |
|-------------------------------------------|---------------------|------|-------------------------------|-----------------------------------------|---------------------|------------------|-------------|
| Polypeptide | Source | PDB | Fold | SCOP-Superfamily | SCOP-Family | SCOP-Superfamily | SCOP-Family |
| Catalase | Proteus Mirabilis | 2cah | Heme linked catalase | | | | |
| Catalase | Cow Liver | 7cat | Heme linked catalase | | | | |
| Catalase | Cow Liver | 8cat | Heme linked catalase | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Polypeptide | Source | PDB | Fold | SCOP-Superfamily | SCOP-Family | SCOP-Superfamily | SCOP-Family |
| 2,5-Diketo-D-Gluconic Acid Reductase | Cornybac terium sp. | 1a80 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase | | |
| 3- α -hydroxysteroid Dehydrogenase | Rat | 1afs | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase | | |

| | | | | | |
|---------------------|---------|-------|-------------------------------|---------------------------------|------------------------|
| Aldehyde Reductase | Pig | 1ae4 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldehyde Reductase | Pig | 1cwn | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldo-keto Reductase | Mouse | 1frb | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 1abn | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 1ads | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Pig | 1ah0 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Pig eye | 1ah3 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Pig | 1ah4 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 1az1 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 1azz2 | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 1mar | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 2accq | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |
| Aldose Reductase | Human | 2acr | β - α TIM Barrel | NAD(P)-linked Oxidoreductase | Aldo-keto Reductase |

| Polypeptide | Source | PDB | Fold | SCOP-Superfamily | SCOP-Family |
|-------------------------|----------------------|------|-------------------------|-------------------------|-------------------------|
| Dihydrofolate Reductase | Candida Albicans | 1ai9 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Candida Albicans | 1aoe | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Pneumocystis carinii | 1daj | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1dlr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1dls | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Chicken Liver | 1dri | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |

| | | | | | |
|-------------------------|----------------------|-------|-------------------------|-------------------------|-------------------------|
| Dihydrofolate Reductase | Chicken Liver | 1dr4 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Chicken Liver | 1dr5 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Chicken Liver | 1dr6 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Chicken Liver | 1dr7 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. coli | 1dre | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. coli | 1drh | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Pneumocystis carinii | 1dryr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1hfp | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1hfq | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1hfr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1ohj | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Human | 1ohk | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. coli | 1ra2 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |

| Dihydrofolate Reductase | E. Coli | 1rb2 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
|------------------------------------------------------------------------------------|---------------------|------|-------------------------------|-------------------------------|------------------------------|
| Dihydrofolate Reductase | E. Coli | 1rh3 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. Coli | 1rx1 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. Coli | 1rx2 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. Coli | 1rx3 | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Lactobacillus casei | 3dfr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | E. Coli | 7dfr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Dihydrofolate Reductase | Chicken Liver | 8dfr | Dihydrofolate Reductase | Dihydrofolate Reductase | Dihydrofolate Reductase |
| Family 7: FAD/NAD (P) Binding Oxidoreductases ('Disulfide Oxidoreductases') | | | | | |
| Polypeptide | Source | PDB | Fold | SCOP-Superfamily | SCOP-Family |
| Glutathione Reductase | E.Coli | 1get | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-linked reductases |
| Glutathione Reductase | E.Coli | 1geu | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-linked reductases |

| Glutathione Reductase | Human | 1grb | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-Linked reductases |
|-------------------------------------------|------------------------|------|-------------------------------|-------------------------------|---------------------------------|
| NADH Peroxidase | Streptococcus faecalis | 2npx | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-Linked reductases |
| Thioredoxin Reductase | E.Coli | 1tdf | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-Linked reductases |
| Trypanothione Reductase* (by active site) | Critchidia fasciculata | 1typ | FAD/NAD (P) Binding Domain | FAD/NAD (P) Binding Domain | FAD/NAD-Linked reductases |
| Family 8: Ferrodoxin-like | | | | | |
| Polypeptide | Source | PDB | Fold | SCOP-Superfamily | SCOP-Family |
| Ferrodoxin Reductase | Pea | 1qga | Ferrodoxin like | Ferrodoxin like | Reductases |
| P450 Reductase | Rat | - | Ferrodoxin like | Ferrodoxin like | NADPH-cytochrome P450 reductase |

The results shown in Table 11 demonstrate that bound conformation of NAD(P)(H) can be correlated with protein fold. Grouping oxidoreductases into pharmacofamilies based on the bound conformations of 5 NAD(P)(H) resulted in a correlation with protein fold. Pharmacofamilies 1-3 consist of polypeptides having the NAD(P)(H) binding Rossman fold. Pharmacofamily 4 consists of polypeptides having heme-linked catalase fold. Pharmacofamily 5 consists of polypeptides having 10 the β - α TIM barrel fold. Pharmacofamily 6 consists of polypeptides having the dihydrofolate reductase fold. Pharmacofamily 7 consists of polypeptides having the FAD/NAD(P)(H) binding domain fold. Trypanathione reductase was added to family 7 by homology of its active 15 site to the active sites of other members of pharmacofamily 7 independent of bound ligand conformation. Pharmacofamily 8 consists of polypeptides having the ferrodoxin like fold. Pharmacofamilies 1 and 2 were identified based on *anti* or *syn* conformation, 20 respectively, of the nicotinamide ring relative to the ribose. Additionally, a change in the torsion angles in the bonds connecting the adenine ribose to the adenine phosphate separates the family members having a Rossman fold into a third pharmacofamily, identified as 25 pharmacofamily 3.

The results described in this example demonstrate that a bound conformation of a ligand can be correlated with polypeptide fold. Furthermore, the results obtained by the method are consistent with 30 results obtained by SCOP. Therefore, classification based on bound conformation of ligands can be used to classify polypeptides according to structure.

EXAMPLE III

**Determination of a conformer model and pharmacophore for
pharmacoclusters 1-8**

This example demonstrates determination of the
5 average bound conformations from pharmacoclusters 1-8 and
construction of conformer models based on the average
bound conformations. This example also demonstrates
construction of a pharmacophore model based on the
average bound conformations and interactions with
10 polypeptides.

Conformer models for each pharmacocluster were
produced by determining an average structure for the
subset of members of each pharmacocluster as described in
Example I. The coordinates for conformer models of
15 pharmacoclusters 1-8 are shown in Part C of Tables 3-10
respectively.

Pharmacophore models were constructed by
aligning the active sites of a pharmacofamily of
oxidoreductases. Three-dimensional overlays were
20 achieved using Insight II overlay module to overlay the
NAD(P) ligands of each enzyme-ligand complex.
Heteroatoms in the surrounding protein that could
function as hydrogen bond acceptors or hydrogen bond
donors were identified in each complex that made
25 interactions with the NAD(P) ligand. These heteroatoms
that had common positions in three dimensional space
(within 3Å of each other in the overlay) in each enzyme
complex and that made a common interaction with the
ligand were then grouped together and tabulated for
30 pharmacophore construction. Water molecules were

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similarly identified and grouped. The grouped heteroatoms and water molecules are listed in Part D of Tables 3-10 below. Finally the average coordinates and the standard deviation for each interaction group were 5 calculated. The final pharmacophore model was produced by overlaying interaction groups on the conformer model (average ligand structure).

The coordinates for pharmacophore models of pharmacoclusters 1-8 are shown in parts B and C of Tables 10 3-10, respectively. Specifically, each conformer model includes the average NAD(P) coordinates (in part C of each Table) and the pharmacophore model includes both the average NADP coordinates, average water coordinates and the average protein heteroatom coordinates (including 15 coordinates in both part B and C of each Table). An exception is the pharmacophore model derived from pharmacofamily 7 which includes average water coordinates and average protein heteroatom coordinates for all polypeptides listed but has a conformer model derived 20 from NAD(P) bound to each polypeptide listed except trypanathione reductase.

A structural representation of each conformer model with overlayed interaction groups used to determine respective pharmacophore models 1-8 is provided in Figure 25 3. The structures shown in Figure 3 reflect the average NAD(P) coordinates shown in Part C of Tables 3-10 and the coordinates for all interacting groups used to calculate the average water coordinates and the average protein heteroatom coordinates as shown in Part D of Tables 3-10. 30 Hydrogen bond acceptors are labeled with an 'A' followed by a number for each group. These are listed in the

pharmacophore Tables and designated on the pharmacophore figures. Donors are labeled with a 'D'; and water molecules are labeled with a 'W'.

This example demonstrates construction of
5 conformer models based on the bound conformations of
ligands in pharmacoclusters. This example also
demonstrates construction of a pharmacophore model based
on the bound conformations of ligands in pharmacoclusters
and their interactions with polypeptides in their
10 respective pharmacofamilies.

Example IV

Correlation Between the Bound Conformation of Ligands and a Conformation-Dependent Property

This example describes a conformation-dependent
15 property that is correlated with a bound conformation of
a ligand.

A 2D [^1H , ^1H] NOESY spectrum was recorded with a
0.2 ml sample of 1 mM NADP and 200 μM of enzyme 1-deoxy
D-xylulose 5-phosphate reductoisomerase (DOXP). The
20 spectrum was measured with a Bruker DRX700 spectrometer
operating at 700 MHZ ^1H frequency. The total measuring
time was about
12 h.

The spectrum is shown in Figure 4 and atoms are
25 identified according to Figure 2. The relative
intensities of the observed transferred NOEs (trNOEs)
between the ribose proton H-C1'N(NC1') and the protons on

the nicotinamide ring, H-C4N and H-C2N shown in Figure 4, reveal that the NADP adopts a *syn* conformation when bound to the enzyme.

The bound conformations in Pharmacocluster 1
5 and 2 can be distinguished according to *anti* or *syn* conformation, respectively, of the nicotinamide ring relative to the ribose. Therefore, these results demonstrate that the relative intensities of the observed trNOE's between the ribose proton H-C1'N(NC1') and the 10 protons on the nicotinamide ring, H-C4N and H-C2N can provide a conformation dependent property useful in distinguishing members of pharmacoclusters 1 and 2.

Example V

15 **Binding compounds having specificity for one or more polypeptide pharmacofamilies.**

This example demonstrates querying a database of compounds to identify individual compounds having similar conformations. This example also demonstrates 20 preferential binding of a compound to a polypeptide of one pharmacofamily over another.

The TTE0001.001.A07 AND TTE0001.002.D02 compounds were identified by using the THREEDOM algorithm to query a database of commercially available molecules 25 (ASINEX; Moscow, Russia) by shape matching with cibacron blue. Coordinates of cibacron blue were obtained from the published 3D structure (Li et al., Proc. Natl. Acad. Sci. USA 92:8846-8850 (1995)). The database was created by converting an SD format file of structures from ASINEX 30 to INTERCHEM format coordinates using the batch2to3

program. Cibacron blue was compared against each structure in the database in multiple orientations to generate a matching score. Out of 37,926 structures searched, the 750 best matching scores were selected.

- 5 From these 750 structures, TTE0001.001.A07 AND
TTE0001.002.D02 were selected and purchased based on
objective criteria such as likely favorable binding
interactions, pharmacophore properties, synthetic
accessibility and likely pharmacokinetic, toxicological,
10 adsorption and metabolic properties.

Kinetic studies were carried out in 1-cm cuvettes in a 1 mL volume at 25°C. Lactate dehydrogenase reactions were monitored spectrophotometrically with a

- 15 Cary 300 by following the decrease in absorbance at 340 nm due to the oxidation of NADH by pyruvate. Lactate dehydrogenase reaction mixtures contained 100 mM Hepes buffer at pH 7.4, as well as 2.5 mM pyruvate, 10 µM NADH, 5 ng/mL lactate dehydrogenase. NADPH, NADH, Hepes
20 buffer, and rabbit muscle lactate dehydrogenase were purchased from Sigma. Cytochrome P450 reductase reactions were monitored by following the decrease in absorbance at 550 nm due to the reduction of ferric cytochrome c by NADPH. Cytochrome P450 reductase
25 reaction mixtures contained 100 mM Hepes buffer at pH 7.4, as well as 80 µM ferric cytochrome c, 10 µM NADPH, and 80 ng/mL cytochrome P450 reductase. Data were fitted using the FORTRAN programs of Cleland, Adv. Enzymol. 45: 273-387 (1977) which perform nonlinear least squares fits
30 to the appropriate equations. Substrates were varied around their Michaelis constants, while nonvaried substrate was kept at a concentration close to its Michaelis constant. The concentration of inhibitor that

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gives 50% inhibition (IC50) values were obtained by fitting data to the equation for a line, where Y values are 1/rate and X values are the concentration of inhibitor, as in a Dixon plot (Segel, *supra*). The X-intercept is the IC50. If a full kinetic profile was done, then K_{is} values were obtained by fitting the data to the equation for a competitive inhibitor:

$$10 \quad \text{rate} = \frac{V_{\max}A}{K_m(1 + I/K_{is}) + A}$$

where rate is the rate of reaction in units of absorbance/minute, V_{\max} is the maximum velocity, K_m is the Michaelis constant for A, K_{is} is the inhibition dissociation constant for the inhibitor, I is the inhibitor concentration, and A is the concentration of NADH or NADPH. In all cases, the fit to the above equation was used only after establishing that the fit to equations for noncompetitive and uncompetitive inhibition were less appropriate based on values for sigma (overall fit) as well as standard deviations for fitted constants (K_{is} and K_{ii}).

As shown in Figure 5, compound TTE0001.001.A07 could inhibit binding of NADH to lactate dehydrogenase and NADPH to cytochrome P450 reductase which are polypeptide members of pharmacofamily 1 and 8 respectively. Compound TTE0001.001.A07 demonstrated high binding affinity for both lactate dehydrogenase and cytochrome P450 reductase.

Analysis of inhibition of binding between NADH and lactate dehydrogenase is shown in Figure 6. Compound TTE0001.002.D02 inhibited lactate dehydrogenase with a K_{is} of 2.1 μ M. Similar measurements of cytochrome P450 reductase with concentrations of compound TTE0001.002.D02 up to 0.5 mM did not indicate inhibition. These results indicated that compound TTE0001.002.D02 had a K_{is} of greater than 1 mM with cytochrome P450 reductase. Thus, compound TTE0001.002.D02 demonstrated preferential binding for pharmacofamily 1 having an inhibitory dissociation constant (K_{is}) that was at least 500 fold lower than for pharmacofamily 8.

The results described in this example demonstrate that a binding compound can be identified by structural comparison to a bound conformation of a ligand. Furthermore, the results demonstrate that binding compounds that interact with polypeptides from multiple pharmacofamilies or compounds that preferentially bind to polypeptides of one pharmacofamily compared to polypeptides of another pharmacofamily can be identified by structural comparison to a bound conformation of a ligand.

Example VI

Identification of a ligand using a pharmacophore model

This example demonstrates construction of a pharmacophore model, use of the model to identify a binding ligand and confirmation of the ability of the identified compound to bind a polypeptide member of the pharmacofamily from which the pharmacophore model was derived.

Pharmacophore models were constructed to include part or all of the NAD(P) shape, hydrogen bond donors, hydrogen bond acceptors and/or other chemical features described in Tables 3-10. The combination of 5 chemical features chosen for each search pharmacophore in a search set were chosen in an attempt to cover a diverse range of combinations of possible chemical interactions and to represent the protein ligand interactions that occur most frequently in the particular 10 pharmacofamily.

Pharmacophore shape was derived using the program CATALYST, and was calculated using the Van der Waals surface for part or all of the structure of the averaged NAD(P) coordinates determined for a 15 pharmacocluster. Desired hydrogen bonding features, water molecules and other chemical motifs were positioned in the pharmacophore model using the average coordinates determined for both the pharmacofamily and pharmacocluster.

20 The components of a pharmacophore model derived from the coordinates presented in Table 3 for pharmacofamily 1 are shown in Figure 7. Figure 7A shows the structure for the conformer model having coordinates listed in Table 3C with a superimposed volume defining 25 the shape of the ligand and indicated by grey spheres. A hydrophobic feature was added to the pharmacophore model at the average position of the hydrophobic region of the nicotinamide ring as shown in Figure 7B. Also shown in Figure 7B is a hydrogen bond acceptor positioned at the 30 average coordinates for the pyrophosphate using the averaged coordinates for the location of hydrogen bond

acceptors utilized in all of the 17 polypeptides of the pharmacofamily. Finally, Figure 7B shows a hydrogen bond donor positioned according to a position where a hydrogen bond donor of a ligand would be expected to have
5 favorable interactions with hydrogen bond acceptors observed in 11 of the polypeptides of pharmacofamily 1. Thus, the hydrogen bond donor does not identify a position of an actual hydrogen bond donor in the NAD(P) ligand, but instead a location to where a potential
10 ligand's hydrogen bond donor could make favorable interactions with the polypeptides of pharmacofamily 1. Figure 7C shows the combined features of figures 7A and 7B present in a pharmacophore model used to search a database of compounds.

15 To identify potential ligands that bind to polypeptides of pharmacofamily 1, computational searches were conducted using CATALYST. Searches were made by comparing the shape and combination of chemical features of the pharmacophore model, shown in Figure 7, to the
20 shape and features of molecules in the database.

An example of a compound identified using the pharmacophore model shown in figure 7C is TTE0008.025.D08. Using a binding assay similar to that described in Example V, compound TTE0008.025.D08 was
25 shown to have inhibitory activity against pharmacofamily 1 member, lactate dehydrogenase ($IC_{50} = 50 \mu M$).

Example VII**Identification of new members of a pharmacofamily using sequence models of pharmacofamilies**

This example demonstrates the construction of
5 Hidden Markov Models based on pharmacofamilies. This
example also demonstrates validation of the Hidden Markov
Models in identifying, from a large sequence database,
members of the pharmacofamilies used to produce the
Hidden Markov Models and new members that were not used
10 to produce the models.

Polypeptides in pharmacofamilies 3 and 5,
respectively, were structurally aligned with PrISM (Yang
& Honig, J Mol Biol. 301:691-711 (2000)). Hidden Markov
Models were produced using the aligned polypeptides of
15 each pharmacofamily as a training set in HMMER 2.1 with
default options (Sean Eddy, unpublished; Department of
Genetics, Washington University, St. Louis). The models
were calibrated using HMMER.

20 The Hidden Markov models were used to search
the PDB for members of the respective pharmacofamilies.
The PDB was used as a test database to validate the
models because there is structural and functional
information about each polypeptide, thereby allowing
25 accurate confirmation of whether a polypeptide identified
with the Hidden Markov Models belongs to a
pharmacofamily.

The PDB sequence library was searched with
Hidden Markov Models using the HMMER 2.1 algorithm.

Polypeptide sequences identified by searching with the Hidden Markov Model were ranked according to an E value score produced by the HMMER program.

The search performed with the Hidden Markov

- 5 Model derived from pharmacofamily 5 returned a set of polypeptides having E values significantly less than 1 as shown in Table 12. Figure 8 shows a plot of $-\ln(E)$ vs. L for the data of Table 12, where L is the location of identified sequences in the list shown in Table 12. Due
- 10 to the low E values, all of the polypeptides shown in Table 12 were compared to a validation set as described below.

TABLE 12: Sequences identified by searching the PDB with a Hidden Markov Model derived from Pharmacofamily 5

| | Sequence | Description | Score | E-value | N |
|----|----------|----------------------------------------------|-------|----------|---|
| 5 | 1el3_A, | Aldose Reductase , mol:protein, length:316 | 774.2 | 2.4e-229 | 1 |
| | 1ads_>, | Aldose Reductase (E.C. 1.1.1.21) Complex, mo | 771.3 | 1.8e-228 | 1 |
| | 2acc_>, | Aldose Reductase (E.C. 1.1.1.21) Wild, mol:p | 771.3 | 1.8e-228 | 1 |
| | 1mar_>, | Aldose Reductase (E.C. 1.1.1.21) -, mol:prot | 771.3 | 1.8e-228 | 1 |
| | 2acr_>, | Aldose Reductase (E.C. 1.1.1.21) Wild, mol:p | 771.3 | 1.8e-228 | 1 |
| 10 | 2acs_>, | Aldose Reductase (E.C. 1.1.1.21) Wild, mol:p | 771.3 | 1.8e-228 | 1 |
| | 1abn_>, | Aldose Reductase (E.C. 1.1.1.21) Mutant, mol | 768.8 | 1e-227 | 1 |
| | 2acu_>, | Aldose Reductase (E.C. 1.1.1.21) Mutant, mol | 764.9 | 1.5e-226 | 1 |
| | 1az1_>, | Aldose Reductase , mol:protein, length:315 | 763.1 | 5.3e-226 | 1 |
| | 1az2_>, | Aldose Reductase , mol:protein, length:315 | 763.1 | 5.3e-226 | 1 |
| 15 | 1ah0_>, | Aldose Reductase , mol:protein-het, length | 760.3 | 3.6e-225 | 1 |
| | 1ah3_>, | Aldose Reductase , mol:protein-het, length | 756.7 | 4.4e-224 | 1 |
| | 1eko_A, | Aldose Reductase , mol:protein-het, length | 756.7 | 4.4e-224 | 1 |
| | 1ah4_, | Aldose Reductase , mol:protein-het, length | 756.7 | 4.4e-224 | 1 |
| | 1d1a_B, | Aldose Reductase (E.C. 1.1.1.21) -, mol:prot | 755.9 | 7.9e-224 | 1 |
| 20 | 1d1a_C, | Aldose Reductase (E.C. 1.1.1.21) -, mol:prot | 755.9 | 7.9e-224 | 1 |
| | 1d1a_D, | Aldose Reductase (E.C. 1.1.1.21) -, mol:prot | 755.9 | 7.9e-224 | 1 |

| | | | | |
|------------|-----------------------------------------------|-------|----------|---|
| 1dla_A, | Aldose Reductase (E.C. 1.1.1.21) -, mol:prot | 755.9 | 7.9e-224 | 1 |
| 1frb_, | Fr-1 Protein , mol:protein, length:315 | 753.0 | 5.8e-223 | 1 |
| 1lwj_B, | 3-Alpha-Hydroxysteroid/Dihydriodiol Dehydroge | 744.3 | 2.4e-220 | 1 |
| 1lwj_A, | 3-Alpha-Hydroxysteroid/Dihydriodiol Dehydroge | 744.3 | 2.4e-220 | 1 |
| 5 1afs_B, | 3-Alpha-Hydroxysteroid Dehydrogenase , mol | 744.3 | 2.4e-220 | 1 |
| 1afs_A, | 3-Alpha-Hydroxysteroid Dehydrogenase , mol | 744.3 | 2.4e-220 | 1 |
| 1c9w_A, | Cho Reductase , mol:protein, length:315 | 728.7 | 1.2e-215 | 1 |
| 1exb_A, | Kv Beta2 Protein , mol:protein, length:332 | 702.6 | 8.9e-208 | 1 |
| 1qrq_B, | Kv Beta2 Protein , mol:protein, length:325 | 693.8 | 3.7e-205 | 1 |
| 10 1qrq_A, | Kv Beta2 Protein , mol:protein, length:325 | 693.8 | 3.7e-205 | 1 |
| 1qrq_D, | Kv Beta2 Protein , mol:protein, length:325 | 693.8 | 3.7e-205 | 1 |
| 1qrq_C, | Kv Beta2 Protein , mol:protein, length:325 | 693.8 | 3.7e-205 | 1 |
| 1ral_-, | 3-Alpha-Hydroxysteroid Dehydrogenase (E.C. 1 | 687.6 | 2.8e-203 | 1 |
| 1a80_-, | 2,5-Diketo-D-Gluconic Acid Reductase A , mol | 555.2 | 2.1e-163 | 1 |
| 15 2alr_-, | Aldehyde Reductase , mol:protein, length:3 | 439.9 | 1e-128 | 1 |
| 1ae4_-, | Aldehyde Reductase , mol:protein, length:3 | 435.5 | 2.2e-127 | 1 |
| 1cwn_-, | Aldehyde Reductase , mol:protein, length:3 | 435.5 | 2.2e-127 | 1 |

The search performed with the Hidden Markov Model derived from pharmacofamily 3 returned a set of polypeptides in which all but one identified polypeptide had an E value significantly less than 1 as shown in 5 Table 13. A significant increase was observed in E value between the penultimate identified polypeptide and last identified polypeptide in the list ordered according to decreasing E value as shown in Table 13. The significant drop position is also evident in a plot of $-\ln(E)$ vs. L 10 as shown in Figure 9. Due to the presence of this large drop position, all polypeptides except the final polypeptide shown in Table 13 were compared to a validation set as described below.

REVIEWER'S COMMENTS

TABLE 13: Sequences identified by searching the PDB with a Hidden Markov Model derived from Pharmacofamily 3: training set 1

| Sequence | Description | Score | E-value | N |
|-------------|------------------------------------------------------------|-------|----------|---|
| 5 1bhs_,' | 17Beta-Hydroxysteroid Dehydrogenase , mol: | 351.9 | 3.2e-102 | 1 |
| 1fds_,' | 17-Beta-Hydroxysteroid-Dehydrogenase , mol | 351.9 | 3.2e-102 | 1 |
| 1fdt_,' | 17-Beta-Hydroxysteroid-Dehydrogenase , mol | 351.9 | 3.2e-102 | 1 |
| 1equ_B,' | Estradiol 17 Beta-Dehydrogenase 1 , mol:prot | 351.9 | 3.2e-102 | 1 |
| 1equ_A,' | Estradiol 17 Beta-Dehydrogenase 1 , mol:prot | 351.9 | 3.2e-102 | 1 |
| 10 1dht_A,' | Estrogenic 17-Beta Hydroxysteroid Dehydrogen | 351.9 | 3.2e-102 | 1 |
| 3dhe_A,' | Estrogenic 17-Beta Hydroxysteroid Dehydrogen | 351.9 | 3.2e-102 | 1 |
| 1iol_,' | Estrogenic 17-Beta Hydroxysteroid Dehydrogen | 351.8 | 3.5e-102 | 1 |
| 1fdt_A,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 1fdv_B,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 15 1fdt_C,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 1fdt_D,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 1fdt_B,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 1fdv_A,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 1fdv_D,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 350.4 | 8.9e-102 | 1 |
| 20 1fdv_C,' | 17-Beta-Hydroxysteroid Dehydrogenase , mol:protein, lenght | 349.4 | 1.9e-101 | 1 |
| lael_A,' | Tropinone Reductase-I , mol:protein, lenght | 349.4 | 1.9e-101 | 1 |

| | | | | |
|------------|---------------------------------------------|-------|----------|---|
| lae1_B, | Tropinone Reductase-I , mol:protein, lenght | 349.4 | 1.9e-101 | 1 |
| 1fdw_, | 17-Beta-Hydroxysteroid Dehydrogenase , mol | 348.7 | 2.9e-101 | 1 |
| 1a27_, | 17-Beta-Hydroxysteroid-Dehydrogenase , mol | 345.7 | 2.4e-100 | 1 |
| 1xe1_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 339.6 | 1.6e-98 | 1 |
| 5 1fdb_>, | Udp-Galactose-4-Epimerase , mol:protein, 1 | 339.6 | 1.6e-98 | 1 |
| 1nai_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 339.6 | 1.6e-98 | 1 |
| 1nah_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 339.6 | 1.6e-98 | 1 |
| luda_, | Udp-Galactose-4-Epimerase , mol:protein, 1 | 339.6 | 1.6e-98 | 1 |
| 1fmc_A, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 10 lahi_B, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 1ahh_B, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 1ahh_A, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 1ahi_A, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 1fmc_B, | 7 Alpha-Hydroxysteroid Dehydrogenase , mol: | 336.9 | 1e-97 | 1 |
| 15 1kvq_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 336.6 | 1.3e-97 | 1 |
| 2udp_B, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 336.2 | 1.7e-97 | 1 |
| 2udp_A, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 336.2 | 1.7e-97 | 1 |
| 1udc_, | Udp-Galactose-4-Epimerase , mol:protein, | 336.2 | 1.7e-97 | 1 |
| 1kvs_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 333.8 | 8.8e-97 | 1 |
| 20 1kvr_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 333.2 | 1.4e-96 | 1 |
| 1kvt_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 332.7 | 2e-96 | 1 |
| 1a9z_, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 331.1 | 5.8e-96 | 1 |

| | | | | |
|------------|----------------------------------------------|-------|---------|---|
| 1kvu_>, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 330.7 | 7.8e-96 | 1 |
| 2hsd_C, | 3 Alpha, 20 Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 2hsd_D, | 3 Alpha, 20 Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 2hsd_B, | 3 Alpha, 20 Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 5 1hdc_B, | 3-Alpha, 20-Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 1hdc_C, | 3-Alpha, 20-Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 1hdc_A, | 3-Alpha, 20-Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 2hsd_A, | 3 Alpha, 20 Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 1hdc_D, | 3-Alpha, 20-Beta-Hydroxysteroid Dehydrogenas | 330.2 | 1.1e-95 | 1 |
| 10 1ybv_A, | Trihydroxynaphthalene Reductase , mol:prot | 328.2 | 4.3e-95 | 1 |
| 1ybv_B, | Trihydroxynaphthalene Reductase , mol:prot | 328.2 | 4.3e-95 | 1 |
| 1a9y_>, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 327.7 | 6.3e-95 | 1 |
| 1bws_A, | GTP-4-Keto-6-Deoxy-D-Mannose Epimerase/Reduc | 321.0 | 6.4e-93 | 1 |
| 1fxs_A, | GTP-Fucose Synthetase , mol:protein, lenght | 320.9 | 6.7e-93 | 1 |
| 15 1bsv_A, | GTP-Fucose Synthetase , mol:protein, lenght | 320.9 | 6.7e-93 | 1 |
| 1gfs_A, | GTP-Fucose Synthetase , mol:protein, lenght | 320.9 | 6.7e-93 | 1 |
| 1cyd_C, | Carbonyl Reductase , mol:protein, length:2 | 306.0 | 2.1e-88 | 1 |
| 1cyd_B, | Carbonyl Reductase , mol:protein, length:2 | 306.0 | 2.1e-88 | 1 |
| 1cyd_D, | Carbonyl Reductase , mol:protein, length:2 | 306.0 | 2.1e-88 | 1 |
| 20 1cyd_A, | Carbonyl Reductase , mol:protein, length:2 | 306.0 | 2.1e-88 | 1 |
| 1bdb_>, | Cis-Biphenyl-2,3-Dihydrodiol-2,3-Dehydrogena | 304.9 | 4.5e-88 | 1 |
| 1enz_>, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 271.5 | 5.3e-78 | 1 |

| | | | | | |
|------------|-----------------------------------------------|---------------------------------------------|---------|---------|---|
| | | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 |
| 1bvr_F, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 1bvr_A, | Enoyl-[Acyl-Carrier-Protein] Reductase , m | 268.4 | 4.3e-77 | 1 | |
| 1zid_>, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 1bvr_B, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 5 1bvr_E, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 1eny_>, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 1bvr_D, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 1bvr_C, | Enoyl-Acyl Carrier Protein (Acp) Reductase, | 268.4 | 4.3e-77 | 1 | |
| 2ae2_B, | Tropinone Reductase-II , mol:protein, leng | 256.7 | 1.5e-73 | 1 | |
| 2ae2_A, | Tropinone Reductase-II , mol:protein, leng | 256.7 | 1.5e-73 | 1 | |
| 2ae1_>, | Tropinone Reductase-II , mol:protein, leng | 256.7 | 1.5e-73 | 1 | |
| 1nas_>, | Sepiapterin Reductase , mol:protein, lengt | 227.9 | 6.7e-65 | 1 | |
| 1oaa_>, | Sepiapterin Reductase , mol:protein, lengt | 227.9 | 6.7e-65 | 1 | |
| 1sep_>, | Sepiapterin Reductase (Dhpr) (E.C. 1..6. | 227.9 | 6.7e-65 | 1 | |
| 15 1dir_D, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 210.6 | 1.1e-59 | 1 | |
| 1dir_A, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 210.6 | 1.1e-59 | 1 | |
| 1dir_B, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 210.6 | 1.1e-59 | 1 | |
| 1dir_C, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 210.6 | 1.1e-59 | 1 | |
| 1dhr_>, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 210.6 | 1.1e-59 | 1 | |
| 20 1hdr_>, | Dihydropteridine Reductase (Dhpr) (E.C. 1..6. | 202.9 | 2.3e-57 | 1 | |
| 1ek6_B, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 120.4 | 1.6e-32 | 1 | |
| 1ek5_A, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 120.4 | 1.6e-32 | 1 | |

| | | | | |
|------------|----------------------------------------------|-------|---------|---|
| 1ek6_A, | Udp-Galactose 4-Epimerase , mol:protein, 1 | 120.4 | 1.6e-32 | 1 |
| 1qsg_G, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1qsg_H, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1d8a_B, | Enoyl-[Acyl-Carrier-Protein] Reductase , m | 94.3 | 1.1e-24 | 1 |
| 5 1c14_A, | Enoyl Reductase , mol:protein, length:262 | 94.3 | 1.1e-24 | 1 |
| 1c14_B, | Enoyl Reductase , mol:protein, length:262 | 94.3 | 1.1e-24 | 1 |
| 1qsg_F, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1qg6_D, | Enoyl-[Acyl-Carrier Protein] Reductase , mo | 94.3 | 1.1e-24 | 1 |
| 1qsg_A, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 10 1qsg_B, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1qsg_C, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1qg6_C, | Enoyl-[Acyl-Carrier Protein] Reductase , mo | 94.3 | 1.1e-24 | 1 |
| 1qsg_E, | Enoyl-Reductase , mol:protein, length:265 | 94.3 | 1.1e-24 | 1 |
| 1qg6_D, | Enoyl-[Acyl-Carrier Protein] Reductase , mo | 94.3 | 1.1e-24 | 1 |
| 15 1qg6_A, | Enoyl-[Acyl-Carrier Protein] Reductase , mo | 94.3 | 1.1e-24 | 1 |
| 1qg6_B, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfi_A, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfi_C, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfi_D, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 20 1dfh_B, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfg_B, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfg_A, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |

| | | | | |
|------------|-----------------------------------------------|-------|---------|---|
| 1d8a_A, | Enoyl-[Acyl-Carrier-Protein] Reductase , m | 94.3 | 1.1e-24 | 1 |
| 1dfi_B, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1dfh_A, | Enoyl Acyl Carrier Protein Reductase, mol:pr | 94.3 | 1.1e-24 | 1 |
| 1cwu_A, | Enoyl Acp Reductase , mol:protein, length:2 | 35.0 | 4.5e-09 | 1 |
| 5 1cwu_B, | Enoyl Acp Reductase , mol:protein, length:2 | 35.0 | 4.5e-09 | 1 |
| 1d7o_A, | Enoyl-[Acyl-Carrier Protein] Reductase (Nadh) | 33.4 | 5.9e-09 | 1 |
| lenp_ , | Enoyl Acyl Carrier Protein Reductase, mol:pr | 33.4 | 5.9e-09 | 1 |
| leno_ , | Enoyl Acyl Carrier Protein Reductase, mol:pr | 33.4 | 5.9e-09 | 1 |
| 1b15_A, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 10 1b15_B, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1a4u_A, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1b14_B, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1a4u_B, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1b14_A, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 15 1b16_A, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1b16_B, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1b21_A, | Alcohol Dehydrogenase , mol:protein, lenght | 28.1 | 1.4e-08 | 1 |
| 1bxk_B, | Dtdp-Glucose 4,6-Dehydratase , mol:protein | -27.4 | 0.00018 | 1 |
| 1bxk_A, | Dtdp-Glucose 4,6-Dehydratase , mol:protein | -27.4 | 0.00018 | 1 |
| 20 1db3_A, | GTP-Mannose 4,6-Dehydratase , mol:protein, | -88.4 | 6 | 1 |

Comparison to a validation set was carried out as follows. The predictive ability of the model was confirmed by comparing the polypeptides identified by the search of the PDB to a validation set including members 5 of the respective pharmacofamily. The ratio of false positives (RFP) and true positives (RTP) was calculated for the set of polypeptides identified from the above described searches. A positive is a polypeptide identified as corresponding to the Hidden Markov Model 10 used. An RFP is the ratio of the number of false positives returned by the search to the number of positives returned by the search, where a false positive is a polypeptide identified as corresponding to the Hidden Markov Model used that is not a member of the 15 validation set. An RTP is the ratio of the number of true positives returned by the search to the number of true positives in the database. Optimal results would have a low RFP and a high RTP.

Comparison of identified polypeptides to the 20 original training set was used to identify new members of pharmacofamily 3. New members can be identified as those having (1) a function similar to members of pharmacofamily 3, (2) a protein fold similar to members of pharmacofamily 3, and/or (3) a bound ligand having a 25 conformation similar to pharmacocluster 3. Polypeptides identified by searching the PDB with pharmacofamily 3 and not present in the training set (training set 1) included Uridine diphosphogalactose-4-epimerase, dTDP-glucose 4,6 dehydratase, GDP-manose 4,6 dehydratase, sulfolipid 30 biosynthesis protein, and alcohol dehydrogenase.

Newly identified members of pharmacofamily 3 were combined with the members of training set 1 to form training set 2. A new sequence model was produced from training set 2 and the PDB searched as described above.

5 A plot of $-\ln(E)$ vs. L for the results of searching the PDB with the sequence model derived from the second pharmacofamily 3 training set is shown in Figure 10. Comparison of the plots in Figures 9 and 10 shows that the second training set, which was improved by adding

10 more members, had a larger difference in E values at the curve inflection occurring just prior to $-\ln(E)=0$, or $E=1$. This statistically significant inflection can be used to identify an E value cutoff of $E=1$.

15 Table 14 shows RTP and RFP values (expressed as percent RFP and percent RTP) obtained for searches of the PDB with Hidden Markov Models derived from pharmacofamilies 5 and the second training set of pharmacofamily 3 and an E value cutoff of 10.

20 Table 14: Results of PDB search with Hidden Markov Models

| pharmacofamily | E value cutoff | RFP % | RTP % |
|--------------------|----------------|-------|-------|
| 3 (training set 2) | 1 | 0 | 100 |
| 3 (training set 2) | 10 | 20 | 100 |
| 5 | 1 | 0 | 100 |
| 25 5 | 10 | 0 | 100 |

As shown in Table 14 the Hidden Markov Models produced from pharmacofamilies 3 and 5 could be used to accurately identify the members of the respective

30 pharmacofamilies in the PDB. Specifically, the Hidden

Markov Models could be used to identify all of the members of the respective pharmacofamilies as indicated by an RTP of 100% and did not falsely identify non-members in the database as indicated by an RFP of 0% with 5 an E value cutoff of 1.

Example VIII

Identification of new members of a pharmacofamily by differential filtering

This example demonstrates the construction of 10 Hidden Markov Models based on different subsets of positions in the structurally aligned members of pharmacofamily 1. In addition, this example demonstrates searching a sequence database by differential filtering and validation of differential filtering in identifying 15 pharmacofamily members in a large sequence database. Furthermore, this example demonstrates identification of a new member of a pharmacofamily using differential filtering.

Polypeptides in pharmacofamily 1 were 20 structurally aligned with PrISM and a first Hidden Markov Model was produced for the aligned polypeptides using HMMER 2.1 as described in Example VII. The training set for the first Hidden Markov Model includes all of the residues shown in Figure 11. The PDB sequence library 25 was searched with the first Hidden Markov Model as described in Example VII.

A second Hidden Markov Model was built to emphasize the binding site region by setting only those residues having at least one atom within 4.5 angstroms of

the binding site as match states. Atoms within 4.5 angstroms of the binding site and used to train the second Hidden Markov model are shown in bold in Figure 11. A SELEX formatted sequence file was generated with 5 HMMER and edited to designate as matched states only the residues having any atom within 4.5 angstroms of the cofactor binding site. Positions not marked as match states by HMMER in the initial generation of the SELEX file, due to insufficient positional population in the 10 alignment, were not marked as match states even if they corresponded to residues close to the cofactor binding site. This sequence file was used (with the --hand option of HMMER) to create a Hidden Markov Model modeling only the sequence motifs. The model was calibrated using 15 HMMER.

The search performed with the first Hidden Markov Model derived from pharmacofamily 1 returned a set of polypeptides having E values in a range including 20 values less than and greater than 1 as shown in Table 15. In contrast to the results presented in Example VII for pharmacofamily 3, a large inflection was not observed in a plot of $-\ln(E)$ versus L as shown in Figure 12. Therefore, differential filtering was used to reduce the 25 ratio of false positives identified while minimizing reduction in the ratio of true positives identified.

TABLE 15: Sequences identified by searching the PDB with a full sequence Hidden Markov Model derived from Pharmacofamily 1

| | Sequence | Description | Score | E-value | N |
|---------|----------------------------------------------|----------------------------------------------|---------|---------|---|
| 5 | 1dxy_>, | D-2-Hydroxyisocaproate Dehydrogenase , mol | 164.5 | 8.4e-46 | 1 |
| 1psd_B, | D-3-Phosphoglycerate Dehydrogenase (Phosphog | 161.9 | 5.1e-45 | 1 | |
| 1psd_A, | D-3-Phosphoglycerate Dehydrogenase (Phosphog | 161.9 | 5.1e-45 | 1 | |
| 2nac_A, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 161.4 | 7.1e-45 | 1 | |
| 2nad_A, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 161.4 | 7.1e-45 | 1 | |
| 10 | 2nac_B, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 161.4 | 7.1e-45 | 1 |
| 2nad_B, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 161.4 | 7.1e-45 | 1 | |
| 91db_A, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 122.4 | 4e-33 | 1 | |
| 91dt_B, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 122.4 | 4e-33 | 1 | |
| 91dt_A, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 122.4 | 4e-33 | 1 | |
| 15 | 91db_B, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 122.4 | 4e-33 | 1 |
| 4mdh_B, | Cytoplasmic Malate Dehydrogenase (E.C. 1, mo | 118.5 | 5.8e-32 | 1 | |
| 4mdh_A, | Cytoplasmic Malate Dehydrogenase (E.C. 1, mo | 118.5 | 5.8e-32 | 1 | |
| 5mdh_A, | Malate Dehydrogenase , mol:protein, length | 116.6 | 2.2e-31 | 1 | |
| 5mdh_B, | Malate Dehydrogenase , mol:protein, length | 116.6 | 2.2e-31 | 1 | |
| 20 | 1bmd_B, | Malate Dehydrogenase (E.C. 1.1.1.37) (Bacter | 113.9 | 1.5e-30 | 1 |
| | 1bmd_A, | Malate Dehydrogenase (E.C. 1.1.1.37) (Bacter | 113.9 | 1.5e-30 | 1 |

| | | | | |
|------------|----------------------------------------------|-------|---------|---|
| 1bdm_B, | Malate Dehydrogenase (E.C. 1.1.1.37) Mutant, | 112.5 | 3.6e-30 | 1 |
| 1bdm_A, | Malate Dehydrogenase (E.C. 1.1.1.37) Mutant, | 112.5 | 3.6e-30 | 1 |
| 1emd_>, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 99.6 | 2.8e-26 | 1 |
| 1cme_>, | Malate Dehydrogenase (E.C. 1.1.1.37) Complex | 99.6 | 2.8e-26 | 1 |
| 5 2cmd_>, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 99.6 | 2.8e-26 | 1 |
| 2ohx_A, | Alcohol Dehydrogenase (Holo Form) (E.C., mol | 98.9 | 4.6e-26 | 1 |
| 1h1d_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Ee, mo | 98.9 | 4.6e-26 | 1 |
| 2ohx_B, | Alcohol Dehydrogenase (Holo Form) (E.C., mol | 98.9 | 4.6e-26 | 1 |
| 2oxi_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Holo,, | 98.9 | 4.6e-26 | 1 |
| 10 1adf_>, | Alcohol Dehydrogenase (E.C. 1.1.1.1) Complex | 98.9 | 4.6e-26 | 1 |
| 1axe_A, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 |
| 1h1d_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Ee, mo | 98.9 | 4.6e-26 | 1 |
| 1adg_>, | Alcohol Dehydrogenase (E.C. 1.1.1.1) Complex | 98.9 | 4.6e-26 | 1 |
| 1adc_B, | Alcohol Dehydrogenase (Adh) (E.C. 1.1.1.1), | 98.9 | 4.6e-26 | 1 |
| 15 1adc_A, | Alcohol Dehydrogenase (Adh) (E.C. 1.1.1.1), | 98.9 | 4.6e-26 | 1 |
| 1adb_B, | Alcohol Dehydrogenase (Adh) (E.C. 1.1.1.1), | 98.9 | 4.6e-26 | 1 |
| 1adb_A, | Alcohol Dehydrogenase (Adh) (E.C. 1.1.1.1), | 98.9 | 4.6e-26 | 1 |
| 1axe_B, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 |
| 11de_D, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 |
| 20 6adh_B, | Holo-Liver Alcohol Dehydrogenase (E.C. 1.1.1 | 98.9 | 4.6e-26 | 1 |
| 11de_B, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 |
| 5adh_>, | Apo-Liver Alcohol Dehydrogenase (E.C. 1.1.1. | 98.9 | 4.6e-26 | 1 |

| | | | | | |
|------------|------------------------------------------------|--------------------------------------------|---------|---------|---|
| | 11dy_D, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 |
| 1bt0_A, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 8adh_8, | Apo-Liver Alcohol Dehydrogenase (E.C. 1.1.99 | 98.9 | 4.6e-26 | 1 | |
| 8adh_9, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 | |
| 11dy_A, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 | |
| 5 11dy_B, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 | |
| 11dy_C, | Alcohol Dehydrogenase , mol:protein, lengt | 98.9 | 4.6e-26 | 1 | |
| 6adh_A, | Holo-Liver Alcohol Dehydrogenase (E.C. 1.1.1.1 | 98.9 | 4.6e-26 | 1 | |
| 1bt0_B, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 1bt0_D, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 10 3bt0_A, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 2oxi_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Holo,, | 98.9 | 4.6e-26 | 1 | |
| 11de_C, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 1bt0_C, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 3bt0_B, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 15 3bt0_D, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 3bt0_C, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 11de_A, | Liver Alcohol Dehydrogenase , mol:protein, | 98.9 | 4.6e-26 | 1 | |
| 1pj_c_A, | L-Alanine Dehydrogenase , mol:protein, len | 98.4 | 6.7e-26 | 1 | |
| 1pj_b_A, | L-Alanine Dehydrogenase , mol:protein, len | 98.4 | 6.7e-26 | 1 | |
| 20 1say_A, | L-Alanine Dehydrogenase , mol:protein, len | 98.4 | 6.7e-26 | 1 | |
| 1axg_D, | Alcohol Dehydrogenase , mol:protein, lengt | 95.9 | 3.6e-25 | 1 | |
| 1axg_C, | Alcohol Dehydrogenase , mol:protein, lengt | 95.9 | 3.6e-25 | 1 | |

| | | | | |
|------------|----------------------------------------------|------|---------|---|
| 1a71_A, | Liver Alcohol Dehydrogenase , mol:protein, | 95.9 | 3.6e-25 | 1 |
| 1a71_B, | Liver Alcohol Dehydrogenase , mol:protein, | 95.9 | 3.6e-25 | 1 |
| 1a72_,' | Horse Liver Alcohol Dehydrogenase , mol:prot | 95.9 | 3.6e-25 | 1 |
| 1axg_A, | Alcohol Dehydrogenase , mol:protein, lenght | 95.9 | 3.6e-25 | 1 |
| 5 1axg_B, | Alcohol Dehydrogenase , mol:protein, lenght | 95.9 | 3.6e-25 | 1 |
| 1b3r_D, | S-Adenosylhomocysteine Hydrolase , mol:pro | 95.9 | 3.8e-25 | 1 |
| 1b3r_A, | S-Adenosylhomocysteine Hydrolase , mol:pro | 95.9 | 3.8e-25 | 1 |
| 1b3r_C, | S-Adenosylhomocysteine Hydrolase , mol:pro | 95.9 | 3.8e-25 | 1 |
| 1b3r_B, | S-Adenosylhomocysteine Hydrolase , mol:pro | 95.9 | 3.8e-25 | 1 |
| 10 1q1j_A, | Alcohol Dehydrogenase , mol:protein, lenght | 95.1 | 6.6e-25 | 1 |
| 1q1h_A, | Alcohol Dehydrogenase , mol:protein, lenght | 95.1 | 6.6e-25 | 1 |
| 1d1s_A, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 1d1s_B, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 1d1t_D, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 15 1d1t_B, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 1d1t_C, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 1agn_C, | Human Sigma Alcohol Dehydrogenase , mol:prot | 93.0 | 2.7e-24 | 1 |
| 1agn_B, | Human Sigma Alcohol Dehydrogenase , mol:prot | 93.0 | 2.7e-24 | 1 |
| 1agn_A, | Human Sigma Alcohol Dehydrogenase , mol:prot | 93.0 | 2.7e-24 | 1 |
| 20 1agn_D, | Human Sigma Alcohol Dehydrogenase , mol:prot | 93.0 | 2.7e-24 | 1 |
| 1d1s_C, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 1d1s_D, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |

| | | | | |
|------------|------------------------------------------------|------|---------|---|
| 1d1t_A, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 93.0 | 2.7e-24 | 1 |
| 7adh_>, | Isonicotinimidylated Liver Alcohol Dehyd , m | 90.9 | 1.2e-23 | 1 |
| 81dh_>, | M=4= Apo-Lactate Dehydrogenase (E.C. 1.1.1, mo | 90.4 | 1.7e-23 | 1 |
| 61dh_>, | M=4= Apo-Lactate Dehydrogenase (E.C. 1.1.1, mo | 90.4 | 1.7e-23 | 1 |
| 5 1ddA_A, | Alcohol Dehydrogenase , mol:protein, lengt | 90.2 | 2e-23 | 1 |
| 1ddA_B, | Alcohol Dehydrogenase , mol:protein, lengt | 90.2 | 2e-23 | 1 |
| 11dm_>, | M=4= Lactate Dehydrogenase (E.C. 1.1.1.27), | 89.9 | 2.5e-23 | 1 |
| 1htb_A, | Beta3 Alcohol Dehydrogenase , mol:protein, | 89.4 | 3.4e-23 | 1 |
| 1htb_B, | Beta3 Alcohol Dehydrogenase , mol:protein, | 89.4 | 3.4e-23 | 1 |
| 10 1deh_B, | Human Beta1 Alcohol Dehydrogenase , mol:prot | 89.4 | 3.4e-23 | 1 |
| 1deh_A, | Human Beta1 Alcohol Dehydrogenase , mol:prot | 89.4 | 3.4e-23 | 1 |
| 3hud_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 89.4 | 3.4e-23 | 1 |
| 3hud_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 89.4 | 3.4e-23 | 1 |
| 1hdz_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 89.4 | 3.4e-23 | 1 |
| 15 1hdy_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-2 | 89.4 | 3.4e-23 | 1 |
| 1hdy_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-2 | 89.4 | 3.4e-23 | 1 |
| 1hdz_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 89.4 | 3.4e-23 | 1 |
| 1hdx_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 89.4 | 3.4e-23 | 1 |
| 20 21db_>, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11dn_D, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11dn_G, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |

Enzyme nomenclature

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| | | | | |
|------------|----------------------------------------------|------|---------|---|
| 11dn_H, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11dn_F, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11dn_E, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11dn_C, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 5 11dn_A, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 11db_ , | Apo-L-Lactate Dehydrogenase (E.C. 1.1.1.27) | 89.3 | 3.5e-23 | 1 |
| 11dn_B, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 89.3 | 3.5e-23 | 1 |
| 51dh_ , | Lactate Dehydrogenase H=4= and S-, mol:prote | 85.4 | 5.2e-22 | 1 |
| 1a7a_B, | S-Adenosylhomocysteine Hydrolase , mol:pro | 83.8 | 1.6e-21 | 1 |
| 10 1a7a_A, | S-Adenosylhomocysteine Hydrolase , mol:pro | 83.8 | 1.6e-21 | 1 |
| 1teh_A, | Human Chichi Alcohol Dehydrogenase , mol:pro | 78.6 | 5.9e-20 | 1 |
| 1teh_B, | Human Chichi Alcohol Dehydrogenase , mol:pro | 78.6 | 5.9e-20 | 1 |
| 1a4i_A, | Methylenetetrahydrofolate Dehydrogenase / Me | 76.0 | 3.6e-19 | 1 |
| 1a4i_B, | Methylenetetrahydrofolate Dehydrogenase / Me | 76.0 | 3.6e-19 | 1 |
| 15 1dib_B, | Methylenetetrahydrofolate Dehydrogenase/Cycl | 76.0 | 3.6e-19 | 1 |
| 1dia_B, | Methylenetetrahydrofolate Dehydrogenase/Cycl | 76.0 | 3.6e-19 | 1 |
| 1dia_A, | Methylenetetrahydrofolate Dehydrogenase/Cycl | 76.0 | 3.6e-19 | 1 |
| 1dib_A, | Methylenetetrahydrofolate Dehydrogenase/Cycl | 76.0 | 3.6e-19 | 1 |
| 1dig_A, | Methylenetetrahydrofolate Dehydrogenase / Cy | 76.0 | 3.6e-19 | 1 |
| 20 1dig_B, | Methylenetetrahydrofolate Dehydrogenase / Cy | 76.0 | 3.6e-19 | 1 |
| 1b8p_A, | Malate Dehydrogenase , mol:protein, length | 73.8 | 1.7e-18 | 1 |
| 1b8v_A, | Malate Dehydrogenase , mol:protein, length | 73.8 | 1.7e-18 | 1 |

| | | | | |
|------------|---------------------------------------------|------|---------|---|
| 1b8u_A, | Malate Dehydrogenase , mol:protein, length | 73.8 | 1.7e-18 | 1 |
| 1bxz_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1ykf_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1bxz_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 5 1bxz_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1ykf_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1ykf_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1bxz_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 1ykf_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 73.4 | 2.2e-18 | 1 |
| 10 lef1_C, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| lef1_B, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| 1qr6_B, | Malic Enzyme 2 , mol:protein-het, length:58 | 71.5 | 8e-18 | 1 |
| lef1_D, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| 1qr6_A, | Malic Enzyme 2 , mol:protein-het, length:58 | 71.5 | 8e-18 | 1 |
| 1do8_A, | Malic Enzyme , mol:protein-het, length:564 | 71.5 | 8e-18 | 1 |
| 1do8_B, | Malic Enzyme , mol:protein-het, length:564 | 71.5 | 8e-18 | 1 |
| lefk_B, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| lefk_D, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| lefk_C, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| 20 1do8_C, | Malic Enzyme , mol:protein-het, length:564 | 71.5 | 8e-18 | 1 |
| 1do8_D, | Malic Enzyme , mol:protein-het, length:564 | 71.5 | 8e-18 | 1 |
| lefk_A, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |

| | | | | |
|------------|-----------------------------------------------|------|---------|---|
| 1ef1_A, | Malic Enzyme , mol:protein-het, length:584 | 71.5 | 8e-18 | 1 |
| 1dld_ , | D-Lactate Dehydrogenase (E.C. 1.1.1.28) Comp | 66.3 | 2.9e-16 | 1 |
| 21dx_ , | Apo-Lactate Dehydrogenase (E.C. 1.1.1.27) , I | 65.6 | 5e-16 | 1 |
| 2d1d_A, | D-Lactate Dehydrogenase , mol:protein, len | 65.5 | 5.2e-16 | 1 |
| 5 2d1d_B, | D-Lactate Dehydrogenase , mol:protein, len | 65.5 | 5.2e-16 | 1 |
| 1hyh_C, | L-2-Hydroxyisocaproate Dehydrogenase , mol | 56.4 | 2.9e-13 | 1 |
| 1hyh_D, | L-2-Hydroxyisocaproate Dehydrogenase , mol | 56.4 | 2.9e-13 | 1 |
| 1hyh_A, | L-2-Hydroxyisocaproate Dehydrogenase , mol | 56.4 | 2.9e-13 | 1 |
| 1hyh_B, | L-2-Hydroxyisocaproate Dehydrogenase , mol | 56.4 | 2.9e-13 | 1 |
| 10 111c_ , | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 48.9 | 5.2e-11 | 1 |
| 1cd0_A, | Alcohol Dehydrogenase , mol:protein, lengt | 46.0 | 4e-10 | 1 |
| 1cd0_B, | Alcohol Dehydrogenase , mol:protein, lengt | 46.0 | 4e-10 | 1 |
| 1a5z_ , | L-Lactate Dehydrogenase , mol:protein, len | 39.2 | 4.4e-08 | 1 |
| 1m1d_B, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 35.8 | 4.6e-07 | 1 |
| 15 1m1d_C, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 35.8 | 4.6e-07 | 1 |
| 1m1d_D, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 35.8 | 4.6e-07 | 1 |
| 1m1d_A, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | 35.8 | 4.6e-07 | 1 |
| 11th_R, | Regular Mixture Of 1:1 Complex, mol:protein, | 31.4 | 9.9e-06 | 1 |
| 111d_B, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) (T-S | 31.4 | 9.9e-06 | 1 |
| 20 111d_A, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) (T-S | 31.4 | 9.9e-06 | 1 |
| 11th_T, | Regular Mixture Of 1:1 Complex, mol:protein, | 31.4 | 9.9e-06 | 1 |
| 1kev_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |

| | | | | |
|------------|----------------------------------------------|------|---------|---|
| 1kev_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 1kev_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 1kev_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 1ped_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 5 1ped_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 1ped_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 1ped_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | 28.9 | 5.3e-05 | 1 |
| 7mdh_C, | Malate Dehydrogenase , mol:protein, length | 26.9 | 0.00012 | 1 |
| 7mdh_A, | Malate Dehydrogenase , mol:protein, length | 26.9 | 0.00012 | 1 |
| 10 7mdh_D, | Malate Dehydrogenase , mol:protein, length | 26.9 | 0.00012 | 1 |
| 7mdh_B, | Malate Dehydrogenase (E.C. 1.1.1.27) M4, mo | 25.7 | 0.00012 | 1 |
| 31dh_ , | Lactate Dehydrogenase, Class II , mol:protei | 23.9 | 0.00015 | 1 |
| 1e3i_B, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 1e3e_A, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 15 1e3i_A, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 1e3e_B, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 1e31_A, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 1e31_B, | Alcohol Dehydrogenase, Class II , mol:protei | 23.9 | 0.00021 | 1 |
| 19dh_B, | D-Glycerate Dehydrogenase (Apo Form) (E.C., | 22.8 | 0.00027 | 1 |
| 20 19dh_A, | D-Glycerate Dehydrogenase (Apo Form) (E.C., | 22.8 | 0.00027 | 1 |
| 1qp8_B, | Formate Dehydrogenase , mol:protein-het, 1 | 21.1 | 0.00038 | 1 |
| 1qp8_A, | Formate Dehydrogenase , mol:protein-het, 1 | 21.1 | 0.00038 | 1 |

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|------------|----------------------------------------------|---------------------|-------|---------|---|
| 1civ_A, | Nadp-Malate Dehydrogenase , | mol:protein, 1 | 20.6 | 0.00042 | 1 |
| 1drv_>, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| 1arz_B, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| 1drz_>, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| 5 1drw_ , | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| larz_A, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| larz_C, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| larz_D, | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| 1dih_ , | Dihydrodipicolinate Reductase , | mol:protein | 18.8 | 0.00062 | 1 |
| 10 1ldg_ , | L-Lactate Dehydrogenase , | mol:protein, len | 2.1 | 0.02 | 1 |
| 1cet_A, | L-Lactate Dehydrogenase , | mol:protein, len | 2.1 | 0.02 | 1 |
| 1ceq_A, | L-Lactate Dehydrogenase , | mol:protein, len | -0.1 | 0.031 | 1 |
| 1d3a_A, | Halophilic Malate Dehydrogenase , | mol:protein | -4.3 | 0.076 | 1 |
| 1d3a_B, | Halophilic Malate Dehydrogenase , | mol:protein | -4.3 | 0.076 | 1 |
| 15 1h1p_B, | Malate Dehydrogenase (E.C. 1.1.1.37) (Haloph | | -4.3 | 0.076 | 1 |
| 1h1p_A, | Malate Dehydrogenase (E.C. 1.1.1.37) (Haloph | | -4.3 | 0.076 | 1 |
| 2h1p_A, | Malate Dehydrogenase , | mol:protein, length | -4.3 | 0.076 | 1 |
| 2h1p_B, | Malate Dehydrogenase , | mol:protein, length | -4.3 | 0.076 | 1 |
| 1b0a_A, | Fold Bifunctional Protein , | mol:protein, 1e | -14.2 | 0.59 | 1 |
| 20 1sdg_ , | Sorbitol Dehydrogenase (E.C. 1.1.1.14) (Theo | | -14.5 | 0.63 | 1 |
| 1gtm_C, | Glutamate Dehydrogenase , | mol:protein, len | -16.4 | 0.94 | 1 |
| 1gtm_A, | Glutamate Dehydrogenase , | mol:protein, len | -16.4 | 0.94 | 1 |

| | | | |
|------------|----------------------------------------------------|------|---|
| 1gtm_B, | Glutamate Dehydrogenase , mol:protein, len -16.4 | 0.94 | 1 |
| 1ges_A, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo -18.0 | 1.3 | 1 |
| 1ges_B, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo -18.0 | 1.3 | 1 |
| 1geu_B, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo -18.0 | 1.3 | 1 |
| 5 1geu_A, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo -18.0 | 1.3 | 1 |
| 3hdh_A, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro -19.8 | 1.9 | 1 |
| 3hdh_B, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro -19.8 | 1.9 | 1 |
| 3hdh_C, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro -19.8 | 1.9 | 1 |
| 1bvu_E, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 10 1bvu_A, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 1bvu_D, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 1bvu_C, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 1bvu_B, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 1bvu_F, | Glutamate Dehydrogenase , mol:protein, len -21.3 | 2.6 | 1 |
| 15 1f0Y_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr -21.6 | 2.7 | 1 |
| 1f0Y_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr -21.6 | 2.7 | 1 |
| 1bz1_B, | Trypanothione Reductase (Oxidized Form) , mo -21.7 | 2.8 | 1 |
| 1bz1_A, | Trypanothione Reductase (Oxidized Form) , mo -21.7 | 2.8 | 1 |
| 1aoq_B, | Trypanothione Reductase , mol:protein, len -21.7 | 2.8 | 1 |
| 20 1aoq_A, | Trypanothione Reductase , mol:protein, len -21.7 | 2.8 | 1 |
| 1f14_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr -21.8 | 2.9 | 1 |
| 1f12_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr -21.8 | 2.9 | 1 |

| | | | | |
|------------|----------------------------------------------|-------|-----|---|
| 1f14_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -21.8 | 2.9 | 1 |
| 1f12_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -21.8 | 2.9 | 1 |
| 1f17_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -21.8 | 2.9 | 1 |
| 1f17_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -21.8 | 2.9 | 1 |
| 5 11pf_B, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4 | -21.9 | 2.9 | 1 |
| 11pf_A, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4 | -21.9 | 2.9 | 1 |
| 1b26_C, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 2tmg_A, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 2tmg_D, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 10 2tmg_B, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b26_A, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 2tmg_E, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b26_B, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b3b_A, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 15 1b3b_C, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b3b_B, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b26_E, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b26_F, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 2tmg_C, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 20 1b3b_D, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 2tmg_E, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b26_D, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |

| | | | | |
|------------|--------------------------------------------------|-------|-----|---|
| 1b3b_E, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 1b3b_F, | Glutamate Dehydrogenase , mol:protein, len | -23.0 | 3.6 | 1 |
| 3had_B, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro | -23.3 | 3.9 | 1 |
| 3had_A, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro | -23.3 | 3.9 | 1 |
| 5 1bxg_B, | Phenylalanine Dehydrogenase , mol:protein, | -23.3 | 3.9 | 1 |
| 1c1d_A, | L-Phenylalanine Dehydrogenase , mol:protein | -23.3 | 3.9 | 1 |
| 1c1x_B, | L-Phenylalanine Dehydrogenase , mol:protein | -23.3 | 3.9 | 1 |
| 1c1x_A, | L-Phenylalanine Dehydrogenase , mol:protein | -23.3 | 3.9 | 1 |
| 1c1d_B, | L-Phenylalanine Dehydrogenase , mol:protein | -23.3 | 3.9 | 1 |
| 10 1bw9_A, | Phenylalanine Dehydrogenase , mol:protein, | -23.3 | 3.9 | 1 |
| 1bw9_B, | Phenylalanine Dehydrogenase , mol:protein, | -23.3 | 3.9 | 1 |
| 1bxg_A, | Phenylalanine Dehydrogenase , mol:protein, | -23.3 | 3.9 | 1 |
| 1ger_B, | Glutathione Reductase (E.C. 1.6.4.2) Complex | -23.3 | 3.9 | 1 |
| 1get_A, | Glutathione Reductase (E.C. 1.6.4.2) Wild-Ty | -23.3 | 3.9 | 1 |
| 15 1get_B, | Glutathione Reductase (E.C. 1.6.4.2) Wild-Ty | -23.3 | 3.9 | 1 |
| 1ger_A, | Glutathione Reductase (E.C. 1.6.4.2) Complex | -23.3 | 3.9 | 1 |
| 1b29_A, | Glutamyl tRNA Reductase , mol:protein, leng | -23.5 | 4.1 | 1 |
| 1b61_>, | Glutamyl tRNA Reductase , mol:protein, leng | -23.5 | 4.1 | 1 |
| 1nda_A, | Trypanothione Oxidoreductase (E.C. 1..6..4..8) | -23.7 | 4.3 | 1 |
| 20 1nda_B, | Trypanothione Oxidoreductase (E.C. 1..6..4..8) | -23.7 | 4.3 | 1 |
| 31ad_A, | Dihydrolipoamide Dehydrogenase (E.C. 1..8..1..4) | -24.4 | 4.9 | 1 |
| 31ad_B, | Dihydrolipoamide Dehydrogenase (E.C. 1..8..1..4) | -24.4 | 4.9 | 1 |

| | | | | | |
|------------|-------------------------|------------------------------|-------|-----|---|
| 2npx_ | Nadh Peroxidase | (E.C. 1.11.1.1) With, mol:pr | -24.4 | 5 | 1 |
| 1joa_ | Nadh Peroxidase | , mol:protein-het, length: | -24.4 | 5 | 1 |
| 1nhq_ | Nadh Peroxidase | (Npx) (E.C. 1.11.1.1), mol:p | -24.4 | 5 | 1 |
| 1npx_ | Nadh Peroxidase | (E.C. 1.11.1.1) Non-Active, | -24.4 | 5 | 1 |
| 5 1nhr_ | Nadh Peroxidase | (Npx) (E.C. 1.11.1.1), mol:p | -24.4 | 5 | 1 |
| 1nhp_ | Nadh Peroxidase | (Npx) (E.C. 1.11.1.1), mol:p | -24.4 | 5 | 1 |
| 1lhs_ | Nadh Peroxidase | (Npx) (E.C. 1.11.1.1), mol:p | -24.4 | 5 | 1 |
| 1qjd_A, | Flavocytochrome C3 | , mol:protein, length:5 | -26.4 | 7.5 | 1 |
| 1e39_A, | Flavocytochrome C3 | , mol:protein, length:5 | -26.4 | 7.5 | 1 |
| 10 1ch6_E, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 1ch6_A, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 1ch6_D, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 1ch6_F, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 1ch6_C, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 15 1ch6_B, | Glutamate Dehydrogenase | , mol:protein, len | -26.8 | 8 | 1 |
| 1bhy_ | P64K | , mol:protein, length:482 | -26.8 | 8.1 | 1 |
| 1objt_ | Surface Protein | , mol:protein, length:482 | -26.8 | 8.1 | 1 |

Differential filtering combining searches with the first Hidden Markov Model and binding site region Hidden Markov Model was performed as follows.

Polypeptides returned from the above described search
5 with the first Hidden Markov Model derived from pharmacofamily 1 and having E values smaller than 1 were combined into a second sequence library. This second sequence library was searched by the binding site region Hidden Markov Model derived from pharmacofamily 1. The 10 set of polypeptides returned from this differential search is shown in Table 16. A plot of $-\ln(E)$ vs. L for the sequences of Table 16 is shown in Figure 13.

TABLE 16: Sequences identified by differential search of the PDB with Hidden Markov Models derived from Pharmacofamily 1 using a 1:1 E value ratio.

| Sequence | Description | Score | E-value | N |
|------------|----------------------------------------------|-------|---------|---|
| 5 2nac_A, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 34.4 | 1.3e-08 | 1 |
| 2nac_B, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 34.4 | 1.3e-08 | 1 |
| 2nad_B, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 34.4 | 1.3e-08 | 1 |
| 2nad_A, | Nad-Dependent Formate Dehydrogenase (E.C. 1. | 34.4 | 1.3e-08 | 1 |
| 1dxy_,' | D-2-Hydroxyisocaproate Dehydrogenase , mol | 26.4 | 3.3e-06 | 1 |
| 10 1psd_A, | D-3-Phosphoglycerate Dehydrogenase (Phosphog | 22.9 | 1e-05 | 1 |
| 1psd_B, | D-3-Phosphoglycerate Dehydrogenase (Phosphog | 22.9 | 1e-05 | 1 |
| 91dt_A, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 14.9 | 0.0001 | 1 |
| 91db_B, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 14.9 | 0.0001 | 1 |
| 91dt_B, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 14.9 | 0.0001 | 1 |
| 15 91db_A, | Lactate Dehydrogenase (E.C. 1.1.1.27) Co, mo | 14.9 | 0.0001 | 1 |
| 8adh_,' | Apo-Liver Alcohol Dehydrogenase (E.C. 1.1.99 | 12.5 | 0.00021 | 1 |
| 1bt0_A, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 11dy_B, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |
| 11dy_A, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |
| 20 1h1d_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Ee, mo | 12.5 | 0.00021 | 1 |
| 11dy_D, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |

| | | | | |
|------------|----------------------------------------------|------|---------|---|
| 11de_D, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 6adh_B, | Holo-Liver Alcohol Dehydrogenase (E.C. 1.1.1 | 12.5 | 0.00021 | 1 |
| 11de_B, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| Sadh_ , | Apo-Liver Alcohol Dehydrogenase (E.C. 1.1.1. | 12.5 | 0.00021 | 1 |
| 5 11de_C, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 3bto_B, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 3bto_D, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 3bto_C, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 11de_A, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 10 6adh_A, | Holo-Liver Alcohol Dehydrogenase (E.C. 1.1.1 | 12.5 | 0.00021 | 1 |
| 1bto_C, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| laxe_B, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |
| 1bto_B, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 1bto_D, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 15 3bto_A, | Liver Alcohol Dehydrogenase , mol:protein, | 12.5 | 0.00021 | 1 |
| 2oxi_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Holo,, | 12.5 | 0.00021 | 1 |
| 11dy_C, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |
| 1hld_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Ee, mo | 12.5 | 0.00021 | 1 |
| laxe_A, | Alcohol Dehydrogenase , mol:protein, lengt | 12.5 | 0.00021 | 1 |
| 20 2oxi_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Holo,, | 12.5 | 0.00021 | 1 |
| 2ohx_B, | (Holo Form) (E.C., mol | 12.5 | 0.00021 | 1 |
| 2ohx_A, | Alcohol Dehydrogenase (Holo Form) (E.C., mol | 12.5 | 0.00021 | 1 |

| | | | | | |
|------------|-----------------------------------------------|------------------------|------|---------|---|
| 1adf_, | Alcohol Dehydrogenase | (E.C. 1.1.1.1) Complex | 12.5 | 0.00021 | 1 |
| 1adb_B, | Alcohol Dehydrogenase (Adh) | (E.C. 1.1.1.1), | 12.5 | 0.00021 | 1 |
| 1adc_A, | Alcohol Dehydrogenase (Adh) | (E.C. 1.1.1.1), | 12.5 | 0.00021 | 1 |
| 1adb_A, | Alcohol Dehydrogenase (Adh) | (E.C. 1.1.1.1), | 12.5 | 0.00021 | 1 |
| 5 1adc_B, | Alcohol Dehydrogenase (Adh) | (E.C. 1.1.1.1), | 12.5 | 0.00021 | 1 |
| 1adg_, | Alcohol Dehydrogenase (E.C. 1.1.1.1) Complex | | 12.5 | 0.00021 | 1 |
| 81dh_>, | M=4= Apo-Lactate Dehydrogenase (E.C. 1.1., mo | | 11.1 | 0.00031 | 1 |
| 61dh_>, | M=4= Apo-Lactate Dehydrogenase (E.C. 1.1., mo | | 11.1 | 0.00031 | 1 |
| 11dm_>, | M=4= Lactate Dehydrogenase (E.C. 1.1.1.27), | | 11.1 | 0.00031 | 1 |
| 10 51dh_' | Lactate Dehydrogenase H=4= and S-, mol:prote | | 10.2 | 0.00039 | 1 |
| 1cmε_' | Malate Dehydrogenase (E.C. 1.1.1.37) Complex | | 10.1 | 0.0004 | 1 |
| 1emd_' | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | | 10.1 | 0.0004 | 1 |
| 2cmd_' | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | | 10.1 | 0.0004 | 1 |
| 1axg_C, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.4 | 0.0005 | 1 |
| 15 1axg_D, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.4 | 0.0005 | 1 |
| 1a71_B, | Liver Alcohol Dehydrogenase , mol:protein, | | 9.4 | 0.0005 | 1 |
| 1a72_>, | Horse Liver Alcohol Dehydrogenase , mol:prot | | 9.4 | 0.0005 | 1 |
| 1axg_A, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.4 | 0.0005 | 1 |
| 1axg_B, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.4 | 0.0005 | 1 |
| 20 1a71_A, | Liver Alcohol Dehydrogenase , mol:protein, | | 9.4 | 0.0005 | 1 |
| 1q1h_A, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.3 | 0.00052 | 1 |
| 1q1j_A, | Alcohol Dehydrogenase , mol:protein, lengt | | 9.3 | 0.00052 | 1 |

| | | | | |
|------------|--------------------------------------------------|-----|---------|---|
| 7adh_>, | Isonicotinimidylated Liver Alcohol Dehyd , m | 8.7 | 0.00061 | 1 |
| 1ddda_B, | Alcohol Dehydrogenase , mol:protein, lengt | 7.5 | 0.00087 | 1 |
| 1ddda_A, | Alcohol Dehydrogenase , mol:protein, lengt | 7.5 | 0.00087 | 1 |
| 1hdY_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-2 | 6.5 | 0.0011 | 1 |
| 5 1hdz_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 3hud_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 1htb_A, | Beta3 Alcohol Dehydrogenase , mol:protein, | 6.5 | 0.0011 | 1 |
| 1htb_B, | Beta3 Alcohol Dehydrogenase , mol:protein, | 6.5 | 0.0011 | 1 |
| 1deh_B, | Human Beta1 Alcohol Dehydrogenase , mol:protein, | 6.5 | 0.0011 | 1 |
| 10 1deh_A, | Human Beta1 Alcohol Dehydrogenase , mol:prot | 6.5 | 0.0011 | 1 |
| 3hud_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 1hdY_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-2 | 6.5 | 0.0011 | 1 |
| 1hdx_B, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 1hdz_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 15 1hdx_A, | Alcohol Dehydrogenase (E.C. 1.1.1.1) (Beta-1 | 6.5 | 0.0011 | 1 |
| 11dn_H, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 11dn_E, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 11dn_F, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 11dn_G, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 20 11dn_C, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 21db_>, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 11dn_D, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |

| | | | | |
|------------|----------------------------------------------|------|--------|---|
| 11dn_A, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 11db_, | Apo-L-Lactate Dehydrogenase (E.C. 1.1.1.27) | 5.6 | 0.0015 | 1 |
| 11dn_B, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 5.6 | 0.0015 | 1 |
| 1d1t_C, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 5 1d1t_B, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1agn_C, | Human Sigma Alcohol Dehydrogenase , mol:prot | 5.1 | 0.0017 | 1 |
| 1d1s_A, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1d1t_D, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1d1s_B, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 10 1agn_B, | Human Sigma Alcohol Dehydrogenase , mol:prot | 5.1 | 0.0017 | 1 |
| 1d1t_A, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1d1s_D, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1d1s_C, | Alcohol Dehydrogenase Class IV Sigma, mol:pr | 5.1 | 0.0017 | 1 |
| 1agn_D, | Human Sigma Alcohol Dehydrogenase , mol:prot | 5.1 | 0.0017 | 1 |
| 15 1agn_A, | Human Sigma Alcohol Dehydrogenase , mol:prot | 5.1 | 0.0017 | 1 |
| 11lc_>, | L-Lactate Dehydrogenase (E.C. 1.1.1.27) Comp | 4.5 | 0.002 | 1 |
| 1bmd_B, | Malate Dehydrogenase (E.C. 1.1.1.37) (Bacter | 3.6 | 0.0027 | 1 |
| 1bmd_A, | Malate Dehydrogenase (E.C. 1.1.1.37) (Bacter | 3.6 | 0.0027 | 1 |
| 1bdm_A, | Malate Dehydrogenase (E.C. 1.1.1.37) Mutant, | 2.5 | 0.0036 | 1 |
| 20 1bdm_B, | Malate Dehydrogenase (E.C. 1.1.1.37) Mutant, | 2.5 | 0.0036 | 1 |
| 1d1d_>, | D-Lactate Dehydrogenase (E.C. 1.1.1.28) Comp | 2.3 | 0.0039 | 1 |
| 2d1d_A, | D-Lactate Dehydrogenase , mol:protein, len | -0.4 | 0.0083 | 1 |

| | | | | |
|------------|----------------------------------------------|------|--------|---|
| 2dld_B, | D-Lactate Dehydrogenase , mol:protein, len | -0.4 | 0.0083 | 1 |
| 1ykf_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1bxz_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1ykf_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 5 1bxz_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1ykf_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1bxz_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1bxz_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 1ykf_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -0.7 | 0.0092 | 1 |
| 10 1hyh_C, | L-2-Hydroxyisocaproate Dehydrogenase , mol: | -0.9 | 0.0096 | 1 |
| 1hyh_D, | L-2-Hydroxyisocaproate Dehydrogenase , mol | -0.9 | 0.0096 | 1 |
| 1hyh_A, | L-2-Hydroxyisocaproate Dehydrogenase , mol | -0.9 | 0.0096 | 1 |
| 1hyh_B, | L-2-Hydroxyisocaproate Dehydrogenase , mol | -0.9 | 0.0096 | 1 |
| 4mdh_B, | Cytoplasmic Malate Dehydrogenase (E.C. 1, mo | -1.3 | 0.011 | 1 |
| 15 5mdh_B, | Malate Dehydrogenase , mol:protein, length | -1.3 | 0.011 | 1 |
| 4mdh_A, | Cytoplasmic Malate Dehydrogenase (E.C. 1, mo | -1.3 | 0.011 | 1 |
| 5mdh_A, | Malate Dehydrogenase , mol:protein, length | -1.3 | 0.011 | 1 |
| 1teh_B, | Human Chichi Alcohol Dehydrogenase , mol:pro | -1.4 | 0.011 | 1 |
| 1teh_A, | Human Chichi Alcohol Dehydrogenase , mol:pro | -1.4 | 0.011 | 1 |
| 20 1b3r_D, | S-Adenosylhomocysteine Hyrolase , mol:pro | -1.7 | 0.012 | 1 |
| 1b3r_B, | S-Adenosylhomocysteine Hyrolase , mol:pro | -1.7 | 0.012 | 1 |
| 1b3r_A, | S-Adenosylhomocysteine Hyrolase , mol:pro | -1.7 | 0.012 | 1 |

| | | | | |
|------------|-----------------------------------------------------|------|-------|---|
| 1b3r_C, | S-Adenosylhomocysteine Hydrolase , mol:protein, len | -1.7 | 0.012 | 1 |
| 1a7a_A, | S-Adenosylhomocysteine Hydrolase , mol:protein, len | -2.3 | 0.014 | 1 |
| 1a7a_B, | S-Adenosylhomocysteine Hydrolase , mol:protein, len | -2.3 | 0.014 | 1 |
| 21dx_,' | Apo-Lactate Dehydrogenase (E.C. 1.1.1.27), I | -2.9 | 0.017 | 1 |
| 5 1say_A, | L-Alanine Dehydrogenase , mol:protein, len | -4.6 | 0.028 | 1 |
| 1pjc_A, | L-Alanine Dehydrogenase , mol:protein, len | -4.6 | 0.028 | 1 |
| 1pjb_A, | L-Alanine Dehydrogenase , mol:protein, len | -4.6 | 0.028 | 1 |
| 1dig_B, | Methylenetetrahydrofolate Dehydrogenase / Cy | -5.1 | 0.032 | 1 |
| 1dib_B, | Methylenetetrahydrofolate Dehydrogenase/Cycl | -5.1 | 0.032 | 1 |
| 10 1dib_A, | Methylenetetrahydrofolate Dehydrogenase/Cycl | -5.1 | 0.032 | 1 |
| 1dig_A, | Methylenetetrahydrofolate Dehydrogenase / Cy | -5.1 | 0.032 | 1 |
| 1dia_B, | Methylenetetrahydrofolate Dehydrogenase/Cycl | -5.1 | 0.032 | 1 |
| 1a4i_B, | Methylenetetrahydrofolate Dehydrogenase / Me | -5.1 | 0.032 | 1 |
| 1a4i_A, | Methylenetetrahydrofolate Dehydrogenase / Me | -5.1 | 0.032 | 1 |
| 15 1dia_A, | Methylenetetrahydrofolate Dehydrogenase/Cycl | -5.1 | 0.032 | 1 |
| 1a5z_,' | L-Lactate Dehydrogenase , mol:protein, len | -5.6 | 0.037 | 1 |
| 31dh_,' | Lactate Dehydrogenase (E.C. 1.1.1.27) M4, mo | -6.3 | 0.045 | 1 |
| 1qp8_B, | Formate Dehydrogenase , mol:protein-het, 1 | -7.7 | 0.067 | 1 |
| 1qp8_A, | Formate Dehydrogenase , mol:protein-het, 1 | -7.7 | 0.067 | 1 |
| 20 1efk_A, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1efl_A, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1efl_C, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |

| | | | | |
|------------|---------------------------------------------|-------|-------|---|
| 1do8_B, | Malic Enzyme , mol:protein-het, length:564 | -7.9 | 0.071 | 1 |
| 1do8_A, | Malic Enzyme , mol:protein-het, length:564 | -7.9 | 0.071 | 1 |
| 1qr6_A, | Malic Enzyme 2 , mol:protein-het, length:58 | -7.9 | 0.071 | 1 |
| 1qr6_B, | Malic Enzyme 2 , mol:protein-het, length:58 | -7.9 | 0.071 | 1 |
| 5 1ef1_B, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1ef1_D, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1do8_C, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1efk_C, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1do8_D, | Malic Enzyme , mol:protein-het, length:564 | -7.9 | 0.071 | 1 |
| 10 1efk_D, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1efk_B, | Malic Enzyme , mol:protein-het, length:584 | -7.9 | 0.071 | 1 |
| 1b8u_A, | Malate Dehydrogenase , mol:protein, length | -9.4 | 0.11 | 1 |
| 1b8v_A, | Malate Dehydrogenase , mol:protein, length | -9.4 | 0.11 | 1 |
| 1b8p_A, | Malate Dehydrogenase , mol:protein, length | -9.4 | 0.11 | 1 |
| 15 1cd0_A, | Alcohol Dehydrogenase , mol:protein, length | -10.4 | 0.15 | 1 |
| 1cd0_B, | Alcohol Dehydrogenase , mol:protein, length | -10.4 | 0.15 | 1 |
| 1ceq_A, | L-Lactate Dehydrogenase , mol:protein, len | -10.6 | 0.15 | 1 |
| 1arz_C, | Dihydrodipicolinate Reductase , mol:protei | -10.6 | 0.16 | 1 |
| 1dih_>, | Dihydrodipicolinate Reductase , mol:protei | -10.6 | 0.16 | 1 |
| 20 1arz_D, | Dihydrodipicolinate Reductase , mol:protei | -10.6 | 0.16 | 1 |
| 1drv_>, | Dihydrodipicolinate Reductase , mol:protei | -10.6 | 0.16 | 1 |
| 1drw_, | Dihydrodipicolinate Reductase , mol:protei | -10.6 | 0.16 | 1 |

| | | | | | | |
|------------|----------------------------------------------|-------|------------------|-------|------|---|
| larz_B, | Dihydrodipicolinate Reductase | , | mol:protein | -10.6 | 0.16 | 1 |
| 1dru_>, | Dihydrodipicolinate Reductase | , | mol:protein | -10.6 | 0.16 | 1 |
| 1arz_A, | Dihydrodipicolinate Reductase | , | mol:protein | -10.6 | 0.16 | 1 |
| 1gdf_B, | D-Glycerate Dehydrogenase (Apo Form) (E.C., | , | mol:protein | -11.1 | 0.18 | 1 |
| 5 1gdh_A, | D-Glycerate Dehydrogenase (Apo Form) (E.C., | , | mol:protein | -11.1 | 0.18 | 1 |
| 1cet_A, | L-Lactate Dehydrogenase | , | mol:protein, len | -11.2 | 0.19 | 1 |
| 1ldg_>, | L-Lactate Dehydrogenase | , | mol:protein, len | -11.2 | 0.19 | 1 |
| 1e3e_B, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 1e3l_B, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 10 1e3l_A, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 1e3i_B, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 1e3i_A, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 1e3e_A, | Alcohol Dehydrogenase, Class II | , | mol:protein | -11.4 | 0.2 | 1 |
| 1get_A, | Glutathione Reductase (E.C. 1.6.4.2) Wild-Ty | -12.1 | 0.24 | 1 | | |
| 15 1ger_B, | Glutathione Reductase (E.C. 1.6.4.2) Complex | -12.1 | 0.24 | 1 | | |
| 1ger_A, | Glutathione Reductase (E.C. 1.6.4.2) Complex | -12.1 | 0.24 | 1 | | |
| 1get_B, | Glutathione Reductase (E.C. 1.6.4.2) Wild-Ty | -12.1 | 0.24 | 1 | | |
| 1ges_B, | Glutathione Reductase (E.C. 1.6.4.2) Complex | -12.1 | 0.24 | 1 | | |
| 1ges_A, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo | -12.1 | 0.24 | 1 | | |
| 20 1geu_A, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo | -12.1 | 0.24 | 1 | | |
| 1geu_B, | Glutathione Reductase (E.C. 1.6.4.2) Nad, mo | -12.1 | 0.24 | 1 | | |
| 1mlnd_B, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | -12.7 | 0.29 | 1 | | |

| | | | | |
|------------|-----------------------------------------------------|-------|------|---|
| 1m1d_D, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | -12.7 | 0.29 | 1 |
| 1m1d_C, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | -12.7 | 0.29 | 1 |
| 1m1d_A, | Malate Dehydrogenase (E.C. 1.1.1.37) -, mol: | -12.7 | 0.29 | 1 |
| 1d3a_B, | Halophilic Malate Dehydrogenase , mol:proto | -15.4 | 0.61 | 1 |
| 5 1h1p_A, | Malate Dehydrogenase (E.C. 1.1.1.37) (Haloph | -15.4 | 0.61 | 1 |
| 2h1p_B, | Malate Dehydrogenase , mol:protein, length | -15.4 | 0.61 | 1 |
| 2h1p_A, | Malate Dehydrogenase , mol:protein, length | -15.4 | 0.61 | 1 |
| 1d3a_A, | Halophilic Malate Dehydrogenase , mol:proto | -15.4 | 0.61 | 1 |
| 1h1p_B, | Malate Dehydrogenase (E.C. 1.1.1.37) (Haloph | -15.4 | 0.61 | 1 |
| 10 1ped_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1kev_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1kev_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1ped_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1kev_D, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 15 1kev_A, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1ped_B, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 1ped_C, | Nadp-Dependent Alcohol Dehydrogenase , mol: | -16.0 | 0.74 | 1 |
| 11pf_A, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4 | -16.5 | 0.85 | 1 |
| 11pf_B, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4) (Theo | -16.5 | 0.85 | 1 |
| 20 1sdg_>, | Sorbitol Dehydrogenase (E.C. 1.1.1.14) (Theo | -16.9 | 0.95 | 1 |
| 31ad_B, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4 | -17.4 | 1.1 | 1 |
| 31ad_A, | Dihydrolipoamide Dehydrogenase (E.C. 1.8.1.4 | -17.4 | 1.1 | 1 |

| | | | | | |
|-------------|----------------------------------------------|----------------------------------------------|-------|-----|---|
| | | Nadh Peroxidase (Npx) (E.C. 1.11.1.1), mol:p | -19.7 | 2.1 | 1 |
| 1nhp_,' | Nadh Peroxidase (E.C. 1.11.1.1) With, mol:pr | -19.7 | 2.1 | 1 | |
| 2npx_,' | Nadh Peroxidase , mol:protein-het, length: | -19.7 | 2.1 | 1 | |
| 1joa_,' | Nadh Peroxidase (Npx) (E.C. 1.11.1.1), mol:p | -19.7 | 2.1 | 1 | |
| 1lhs_,' | Nadh Peroxidase (Npx) (E.C. 1.11.1.1), mol:p | -19.7 | 2.1 | 1 | |
| 5 1nhr_,' | Nadh Peroxidase (E.C. 1.11.1.1) Non-Active, | -19.7 | 2.1 | 1 | |
| 1npx_,' | Nadh Peroxidase (Npx) (E.C. 1.11.1.1), mol:p | -19.7 | 2.1 | 1 | |
| 1nhq_,' | Glutamate Dehydrogenase , mol:protein, len | -19.7 | 2.1 | 1 | |
| 1gtnm_C, | Glutamate Dehydrogenase , mol:protein, len | -19.9 | 2.2 | 1 | |
| 1gtnm_B, | Glutamate Dehydrogenase , mol:protein, len | -19.9 | 2.2 | 1 | |
| 10 1gtnm_A, | Glutamate Dehydrogenase , mol:protein, len | -19.9 | 2.2 | 1 | |
| 1bw9_B, | Phenylalanine Dehydrogenase , mol:protein, | -20.3 | 2.5 | 1 | |
| 1bxg_B, | Phenylalanine Dehydrogenase , mol:protein, | -20.3 | 2.5 | 1 | |
| 1c1d_A, | L-Phenylalanine Dehydrogenase , mol:protein, | -20.3 | 2.5 | 1 | |
| 1bxg_A, | Phenylalanine Dehydrogenase , mol:protein, | -20.3 | 2.5 | 1 | |
| 15 1c1x_B, | L-Phenylalanine Dehydrogenase , mol:protein | -20.3 | 2.5 | 1 | |
| 1c1x_A, | L-Phenylalanine Dehydrogenase , mol:protein | -20.3 | 2.5 | 1 | |
| 1bw9_A, | L-Phenylalanine Dehydrogenase , mol:protein, | -20.3 | 2.5 | 1 | |
| 3had_A,' | L-3-Hydroxyacyl'Coa Dehydrogenase , mol:pro | -21.9 | 3.9 | 1 | |
| 20 3had_B, | L-3-Hydroxyacyl Coa Dehydrogenase , mol:pro | -21.9 | 3.9 | 1 | |
| 1e39_A, | Flavocytochrome C3 , mol:protein, length:5 | -22.2 | 4.3 | 1 | |
| 1qjd_A, | Flavocytochrome C3 , mol:protein, length:5 | -22.2 | 4.3 | 1 | |

TABLE II. SPECTRA

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| | | | | |
|------------|----------------------------------------------|-------|-----|---|
| 1f0y_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.4 | 4.5 | 1 |
| 1f0y_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.4 | 4.5 | 1 |
| 1f14_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| 1f17_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| 5 1f17_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| 1f12_A, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| 1f12_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| 1f14_B, | L-3-Hydroxyacyl-Coa Dehydrogenase , mol:pr | -22.5 | 4.7 | 1 |
| laog_B, | Trypanthione Reductase , mol:protein, len | -22.6 | 4.9 | 1 |
| 10 laog_A, | Trypanthione Reductase , mol:protein, len | -22.6 | 4.9 | 1 |
| 1bz1_B, | Trypanthione Reductase (Oxidized Form) , mo | -22.6 | 4.9 | 1 |
| 1bz1_A, | Trypanthione Reductase (Oxidized Form) , mo | -22.6 | 4.9 | 1 |
| 1bvu_E, | Glutamate Dehydrogenase , mol:protein, len | -23.2 | 5.7 | 1 |
| 1bvu_D, | Glutamate Dehydrogenase , mol:protein, len | -23.2 | 5.7 | 1 |
| 15 1bvu_C, | Glutamate Dehydrogenase , mol:protein, len | -23.2 | 5.7 | 1 |
| 1bvu_B, | Glutamate Dehydrogenase , mol:protein, len | -23.2 | 5.7 | 1 |
| 1bvu_F, | Glutamate Dehydrogenase , mol:protein, len | -23.2 | 5.7 | 1 |
| 1bvu_A, | Nadp-Malate Dehydrogenase , mol:protein, 1 | -23.4 | 6.1 | 1 |
| 20 1nda_B, | Trypanothione Oxidoreductase (E.C. 1.6.4.8) | -23.5 | 6.3 | 1 |
| 1nda_A, | Trypanothione Oxidoreductase (E.C. 1.6.4.8) | -23.5 | 6.3 | 1 |
| 11th_R, | Regular Mixture Of 1:1 Complex, mol:protein, | -23.8 | 6.8 | 1 |

11th T, Regular Mixture Of 1:1 Complex, mol:protein, -23.8 6.8 1
 111d A, L-Lactate Dehydrogenase (E.C. 1.1.1.27) (T-S -23.8 6.8 1
 111d B, L-Lactate Dehydrogenase (E.C. 1.1.1.27) (T-S -23.8 6.8 1
 1objt_, Surface Protein , mol:protein, length:482 -24.5 8.2 1
 5 1bhY_ , P64K , mol :protein, length:482 -24.5 8.2 1

The polypeptides returned from the differential search and having various E value ratios were compared to a validation set as described in Example VII. The RFP% and RTP% obtained for the search based on the full sequence Hidden Markov Model and based on the differential filtering search are shown in Table 17. In Table 17 the first and second rows show the results of searches of the PDB with the first sequence model with E value cutoffs of 1 and 10 respectively. The last two rows show the results of differential filtering in which the sequences identified from a search with the first model (in lines 1 and 2) were searched again with a second model. Specifically, line 3 shows the results of searching the sequences identified from the first model at E=10 with the second model at E=10 and line 4 shows the results of searching the sequences identified from the first model at E=1 with the second model at E=10.

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Table 17: Results of PDB search compared to original validation set

| Search | E value first HMM | E value binding site HMM | E value ratio | RFP% | RTP% |
|---------------------------|----------------------|--------------------------------|------------------|------|------|
| 5 full sequence HMM | 1 | NA | NA | 9 | 100 |
| | differential | 10 | 1:10 | 8 | 99 |
| | full sequence HMM | NA | NA | 48 | 100 |
| | differential | 10 | 1:1 | 39 | 99 |

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As shown in Table 17, differential filtering provided a significant improvement in RFP with little or no effect on the RTP as compared between respective E value cutoffs. The results of Table 17 also show that by 15 adjusting the E value ratios, significantly lower RFP can be achieved with minor effects on the RTP.

Polypeptides identified by differential filtering and not present in a pharmacofamily 1 validation set can be identified as new members of 20 pharmacofamily 1. New members can be identified as those having (1) a function similar to members of pharmacofamily 1, (2) a protein fold similar to members of pharmacofamily 1, and/or (3) a bound ligand having a conformation similar to pharmacocluster 1. By this

criteria polypeptide D-glycerate dehydrogenase was identified as a new member of pharmacofamily 1.

An improvement in the ability of differential filtering to accurately and specifically identify members 5 of pharmacofamily 1 can be achieved by adding newly identified members to the original validation set to create an expanded validation set. Table 18 presents the RFP and RTP values obtained when the polypeptides produced by differential filtering were compared to the 10 expanded validation set containing newly added polypeptide D-glycerate dehydrogenase.

Table 18: Results of PDB search compared to expanded validation set

| | Search | E value first HMM | E value binding site HMM | E value ratio | RFP% | RTP% |
|----|----------------------|----------------------|--------------------------------|------------------|------|------|
| 15 | full sequence HMM | 1 | NA | NA | 3 | 100 |
| | differential | 1 | 10 | 1:10 | 2 | 98 |
| 20 | full sequence HMM | 10 | NA | NA | 45 | 100 |
| | differential | 10 | 10 | 1:1 | 36 | 98 |

Comparison of the results from the original validation set shown in Table 17 with the results from the expanded validation set shown in Table 18 indicate an improvement in RFP with only minor reduction in RTP.

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Example IX

Identification of members of pharmacofamily 1 in the TB proteome

This example demonstrates searching the TB proteome with full sequence Hidden Markov Models derived 10 from various pharmacofamilies. This example demonstrates identification of potential functions for sequences in a proteome for which a function has not yet been assigned. This example also demonstrates determination of which pharmacofamily a newly identified sequence most likely 15 belongs.

Full sequence Hidden Markov Models were produced for pharmacofamilies 1, 2, 3, 5, & 6 as described in Example VII. The full sequence Hidden Markov Models were used for single sequence searches of 20 the TB proteome essentially as described in Example VII. The TB proteome has been described in Cole et al., Nature 393:537-544 (1998).

The results of a search with the full sequence Hidden Markov Model derived from pharmacofamily 1 is 25 shown in Table 20. As shown in Table 20 a number of "putative" or "probable" dehydrogenase sequences were identified in the proteome having relatively low E values. Examples of these dehydrogenases are indicated in bold font in Table 20. Thus, indicating that a

sequence model derived from a pharmacofamily can be used to identify potential new members of a protein family in a proteome containing sequences encoding polypeptides of unknown function.

TABLE 20: Sequences identified from a search of the TB proteome with the full sequence Hidden Markov Model derived from pharmacofamily 1

| | Sequence | Description | Score | E-value | N |
|----|----------|-----------------------------------------------|-------|---------|---|
| 5 | Rv2996c, | D-3-phosphoglycerate dehydrogenase, TB.seq, | 80.6 | 2.2e-21 | 1 |
| | Rv0728c, | similar to D-3-phosphoglycerate dehydrogenas | 53.7 | 2.7e-13 | 1 |
| | Rv1240, | malate dehydrogenase, TB.seq, 1383211:138419 | 45.3 | 9e-11 | 1 |
| | Rv3248c, | adenosylhomocysteinase, TB.seq, 3628159:3629 | 40.5 | 2.5e-09 | 1 |
| | Rv2780, | L-alanine dehydrogenase, TB.seq, 3086817:308 | 18.8 | 8.9e-05 | 1 |
| 10 | Rv3356c, | methylenetetrahydrofolate dehydrogenase, TB. | 18.6 | 9.3e-05 | 1 |
| | Rv0155, | pyridine transhydrogenase subunit , TB.seq, | 8.5 | 0.00075 | 1 |
| | Rv2259, | putative alcohol dehydrogenase (Zn dependent | 2.6 | 0.0026 | 1 |
| | Rv0761c, | zinc-containing alcohol dehydrogenase, TB.se | 0.1 | 0.0043 | 1 |
| | Rv2332, | probable malate oxidoreductase, TB.seq, 2604 | -3.1 | 0.0085 | 1 |
| 15 | Rv3141, | 3-hydroxyacyl-CoA dehydrogenase, TB.seq, 350 | -8.0 | 0.023 | 1 |
| | Rv2048c, | polyketide synthase (erythronolides synthase- | -8.8 | 0.028 | 1 |
| | Rv3726, | Putative alcohol dehydrogenase, zinc-type, T | -10.1 | 0.036 | 1 |
| | Rv1895, | similar to sorbitol and alcohol dehydrogenas | -11.2 | 0.046 | 1 |
| | Rv0509, | glutamyl-tRNA reductase, TB.seq, 600439:6018 | -11.8 | 0.052 | 1 |
| 20 | Rv0688, | putative oxidoreductase, TB.seq, 787938:7891 | -12.9 | 0.065 | 1 |
| | Rv1527c, | polyketide synthase, TB.seq, 17220084:1728407 | -13.9 | 0.079 | 1 |

| | | | | | | |
|----|----------|----------------------------------------------|--------------------------|-------|------|---|
| | Rv1175c, | 2,4-Dienoyl-CoA Reductase, TB.seq, | 1306203:1 | -15.1 | 0.1 | 1 |
| | Rv3777, | 3-Hydroxyacyl-CoA Dehydrogenase, TB.seq, | 422 | -16.9 | 0.15 | 1 |
| | Rv0162c, | alcohol dehydrogenase (Zn), TB.seq, | 191985:1 | -18.1 | 0.19 | 1 |
| | Rv0149, | putative oxidoreductase, TB.seq, | 175698:1766 | -18.6 | 0.21 | 1 |
| 5 | Rv3436c, | glucosamine-fructose-6-phosphate aminotransf | -19.4 | 0.25 | 1 | |
| | Rv3086, | zinc-containing alcohol dehydrogenase, TB.se | -19.4 | 0.25 | 1 | |
| | Rv2933, | phenolphthiocerol synthesis (pkSD), TB.seq, | 3 | -21.0 | 0.34 | 1 |
| | Rv0886, | ferredoxin, ferredoxin-NADP reductase, TB.se | -21.2 | 0.36 | 1 | |
| | Rv1869c, | probable reductase (like rhodocoxin reductas | -22.0 | 0.42 | 1 | |
| | Rv3468c, | dTDP-glucose 4,6-dehydratase, TB.seq, | 388497 | -22.4 | 0.46 | 1 |
| 10 | Rv1543, | probable fatty-acyl CoA reductase, TB.seq, | 1 | -23.7 | 0.61 | 1 |
| | Rv0892, | putative monooxygenase, TB.seq, | 993851:99533 | -24.7 | 0.75 | 1 |
| | Rv0104, | , TB.seq, | 122315:123826, MW:53420. | -24.7 | 0.76 | 1 |
| | Rv2381c, | mycobactin/exochelin synthesis (polyketide s | -25.2 | 0.82 | 1 | |
| 15 | Rv0242c, | 3-oxoacyl-[ACP] reductase, TB.seq, | 290666:29 | -25.9 | 0.95 | 1 |
| | Rv0952, | succinyl-CoA synthase , TB.seq, | 1063138:1064 | -26.1 | 1 | |
| | Rv1662, | polyketide synthase, TB.seq, | 1881702:1886507 | -26.5 | 1.1 | 1 |
| | Rv3858c, | small subunit of NADH-dependent glutamate sy | -26.5 | 1.1 | 1 | |
| | Rv3391, | fatty acyl-CoA reductase, TB.seq, | 3805617:38 | -26.6 | 1.1 | 1 |
| 20 | Rv3057c, | possible ketoacyl reductase, TB.seq, | 3417799 | -27.0 | 1.2 | 1 |
| | Rv3860, | (35.2% id) , TB.seq, | 4336774:4337943, MW:421 | -27.0 | 1.2 | 1 |
| | Rv0462, | probable dihydrolipoamide dehydrogenase, TB. | -27.1 | 1.2 | 1 | |

| | | | | | | |
|----|----------|------------------------------------------------|-----------------|-------|-----|---|
| | Rv2766c, | 3-oxoacyl-[ACP] reductase, TB.seq, | 3075588:3 | -27.7 | 1.4 | 1 |
| | Rv3559c, | short-chain alcohol dehydrogenase, TB.seq, | 3 | -28.0 | 1.5 | 1 |
| | Rv3895c, | , TB.seq, 4380453:4381937, MW:51588. | | -28.6 | 1.7 | 1 |
| | Rv0860, | , TB.seq, 956291:958450, MW:76105. | | -28.8 | 1.7 | 1 |
| 5 | Rv1661, | polyketide synthase, TB.seq, | 1875302:1881679 | -28.9 | 1.8 | 1 |
| | Rv3660c, | involved in differentiation inhibition between | | -29.0 | 1.8 | 1 |
| | Rv1739c, | possible sulphate transporter, TB.seq, | 19659 | -29.3 | 1.9 | 1 |
| | Rv1279, | probable choline dehydrogenase, TB.seq, | 1430 | -29.3 | 2 | 1 |
| | Rv0794c, | dihydrolipoamide dehydrogenase, TB.seq, | 8871 | -29.6 | 2.1 | 1 |
| 10 | Rv2072c, | probable methyltransferase, TB.seq, | 2328975: | -29.6 | 2.1 | 1 |
| | Rv3302c, | glycerol-3-phosphate dehydrogenase, TB.seq, | | -29.7 | 2.1 | 1 |
| | Rv3158, | NADH dehydrogenase chain N, TB.seq, | 3525787: | -30.0 | 2.3 | 1 |
| | Rv1865c, | short-chain alcohol dehydrogenase, TB.seq, | 2 | -30.0 | 2.3 | 1 |
| | Rv2202c, | carbohydrate kinase, TB.seq, | 2467054:2468025 | -30.2 | 2.3 | 1 |
| 15 | Rv1496, | YPLE_CAUCR P37895 & ; Q05072, TB.seq, | 1686 | -30.5 | 2.5 | 1 |
| | Rv0037c, | probable membrane protein, TB.seq, | 39880:412 | -31.1 | 2.8 | 1 |
| | Rv3485c, | short-chain alcohol dehydrogenase family, TB | | -31.2 | 2.9 | 1 |
| | Rv3072c, | similar to alkanal monooxygenase beta chains | | -31.3 | 3 | 1 |
| | Rv3825c, | polyketide synthase, TB.seq, | 4293225:4299602 | -31.6 | 3.2 | 1 |
| 20 | Rv1272c, | probable ABC transporter, TB.seq, | 1420411:14 | -32.3 | 3.6 | 1 |
| | Rv1245c, | putative dehydrogenase, TB.seq, | 1387799:1388 | -32.3 | 3.6 | 1 |
| | Rv1350, | 3-oxoacyl-[ACP] reductase, TB.seq, | 1517489:1 | -32.3 | 3.7 | 1 |

| | | | | |
|-------------|----------------------------------------------|-------|-----|---|
| Rv3045, | alcohol dehydrogenase, TB.seq, 3406282:34073 | -32.5 | 3.8 | 1 |
| Rv2946c, | polyketide synthase, TB.seq, 3291503:3296350 | -32.6 | 3.8 | 1 |
| Rv3382c, | LytB protein homologue, TB.seq, 3796447:3797 | -32.6 | 3.8 | 1 |
| Rv2787, | , TB.seq, 3095108:3096868, MW:63850. | -33.1 | 4.3 | 1 |
| 5 Rv2940c, | mycocerosic acid synthase, TB.seq, 3276380:3 | -33.3 | 4.5 | 1 |
| Rv3728, | possible sugar transporter, TB.seq, 4174870: | -33.4 | 4.6 | 1 |
| Rv0178, | , TB.seq, 208936:209667, MW:25880. | -33.6 | 4.8 | 1 |
| Rv3395c, | , TB.seq, 3811021:3811902, MW:29873. | -33.7 | 4.9 | 1 |
| Rv1743, | serine-threonine protein kinase, TB.seq, 196 | -33.8 | 4.9 | 1 |
| 10 Rv3727, | similar to phytoene dehydrogenase precursor, | -33.8 | 5 | 1 |
| Rv2855, | glutathione reductase homologue, TB.seq, 316 | -34.0 | 5.1 | 1 |
| Rv1405c, | similar to phosphatidylethanolamine N-methyl | -34.0 | 5.2 | 1 |
| Rv1294, | homoserine dehydrogenase, TB.seq, 1449373:14 | -34.2 | 5.4 | 1 |
| Rv3709c, | aspartokinase, TB.seq, 4152218:4153480, MW:4 | -34.2 | 5.4 | 1 |
| 15 Rv2006, | trehalose-6-phosphate phosphatase, TB.seq, 2 | -34.3 | 5.5 | 1 |
| Rv1069c, | v sim to B1306.04c, hydrophobic N-term regio | -34.6 | 5.8 | 1 |
| Rv1714, | Probable oxidoreductase/gluconate 3-dehydrog | -34.8 | 6.1 | 1 |
| Rv0782, | protease II, a subunit, TB.seq, 874730:87638 | -34.8 | 6.1 | 1 |
| Rv3116, | molybdopterin biosynthesis, TB.seq, 3482773: | -35.0 | 6.3 | 1 |
| 20 Rv2062c, | cobalt insertion , TB.seq, 2317170:2320751, | -35.0 | 6.4 | 1 |
| Rv1621c, | ABC transporter, TB.seq, 1821691:1823271, MW | -35.0 | 6.4 | 1 |
| Rv2214c, | probable epoxide hydrolase , TB.seq, 2479924 | -35.0 | 6.4 | 1 |

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| Rv2713, | probable dehydrogenase, TB.seq, | 3025438:3026 | -35.1 | 6.4 | 1 |
|-------------|----------------------------------------------|-----------------------------|-------|-----|---|
| Rv3700c, | probable acetyltransferase, TB.seq, | 4142748: | -35.2 | 6.6 | 1 |
| Rv0113, | phosphohexitose isomerase, TB.seq, | 137317:137 | -35.2 | 6.6 | 1 |
| Rv2110c, | proteasome , TB.seq, | 2369727:2370599, MW:302 | -35.6 | 7.3 | 1 |
| 5 Rv2380c, | mycobactin/exochelin synthesis (lysine ligat | -35.7 | 7.4 | 1 | |
| Rv0507, | conserved large membrane protein, TB.seq, | 59 | -35.9 | 7.6 | 1 |
| Rv1530, | alcohol dehydrogenase (Zn), TB.seq, | 1731371: | -36.0 | 7.9 | 1 |
| Rv2931, | phenolpthiocerol synthesis (pkSB) , TB.seq, | 3 | -36.1 | 7.9 | 1 |
| Rv2002, | 3-oxoacyl-[ACP] reductase, TB.seq, | 2247658:2 | -36.2 | 8.1 | 1 |
| Rv1300, | protoporphyrinogen oxidase, TB.seq, | 1456563: | -36.6 | 8.8 | 1 |
| 10 Rv2559c, | YCAJ_HAEIN P45262, TB.seq, | 2878572:2879927, | -36.6 | 8.9 | 1 |
| Rv2123, | , TB.seq, | 2381069:2382487 , MW:48532. | -36.7 | 9.1 | 1 |
| Rv1437, | phosphoglycerate kinase, TB.seq, | 1614327:161 | -36.7 | 9.1 | 1 |
| Rv1410c, | probable drug efflux protein, TB.seq, | 158621 | -36.7 | 9.1 | 1 |
| Rv3206c, | probably involved in molybdopterin biosynth | | -36.8 | 9.2 | 1 |
| 15 Rv0209, | , TB.seq, | 249036:250118 , MW:38133. | -37.0 | 9.6 | 1 |
| Rv3106, | adrenodoxin and NADPH ferredoxin reductase, | | -37.1 | 9.8 | 1 |
| Rv3131, | (35.0% id) , TB.seq, | 3496548:3497543, MW:3597 | -37.1 | 9.9 | 1 |

Comparison of the E values obtained for a specific sequence identified from searches with full sequence Hidden Markov Models derived from multiple pharmacofamilies could be used to determine to which 5 pharmacofamily an identified sequence most likely belonged. In a representative result, a sequence in the TB proteome annotated as 'putative dehydrogenase Rv 1245c' was predicted to belong to dehydrogenase pharmacofamily 3 with an E value of 5×10^{-28} and to 10 dehydrogenase pharmacofamily 1 with an E value of 55. According to searches with full sequence Hidden Markov Models derived from pharmacofamilies 2, 5, and 6 there was no significant probability (small enough E value) that the protein belonged to pharmacofamilies 2, 5, or 6. 15 Thus, it was concluded that 'putative dehydrogenase Rv 1245c' is a member of pharmacofamily 3.

These results indicate that it was possible to make a statistically significant prediction about which pharmacofamily 'putative dehydrogenase Rv 1245c' belongs 20 based solely on comparison to sequence models for a variety of pharmacofamilies. Thus, even in the absence of functional characterization of 'putative dehydrogenase Rv 1245c' a ligand geometry can be identified by comparison to pharmacocluster 3 according to the methods 25 described herein. Based on this ligand geometry a binding compound can be identified or designed that will specifically bind to 'putative dehydrogenase Rv 1245c.'

This example demonstrates that, once built and verified, sequence models derived from various 30 pharmacofamilies can be used to provide pharmacofamily annotation of a proteome. Sequences unable to be

adequately annotated by other methods can be identified as members of a pharmacofamily in this way. Furthermore, once identified, polypeptides encoded by newly identified sequences can be targeted with an appropriate binding 5 compound identified or designed based on the appropriate pharmacocluster.

Coordinates for the conformer and pharmacophore models and data used in their construction is presented in Tables 3-10 below. Part A of each Table lists subset 10 of structures used in constructing the model including molecule numbers for cross-referencing between parts A-C, the PDB accession number, the name of the polypeptide, and the RMSD from the pharmacocluster average. Part B of each Table lists the average coordinates for heteroatoms 15 and waters of the pharmacophore model and includes the atom name (cross referenced to part D), designation of interaction ("ACC," acceptor; "DON," donor; and "WAT," water), total number of atoms included in the calculation of the average, and X, Y, Z coordinates with respective 20 standard deviations (σ). Part C of each Table lists the coordinates of the conformer model using the atom designations of Figure 2 and X, Y, Z coordinates with respective standard deviations (σ). Part D of each Table lists the coordinates for interacting molecules used to 25 determine the pharmacophore model including the atom name, residue molecule # (which identifies the residue type and molecule number cross-referenced to Part A), residue number from the PDB structure, total number of atoms summed for the average coordinates, and X, Y, Z 30 coordinates with respective standard deviations (σ). The bolded entries in part D correspond to the average values

reported in part B. Atom names are identified according to IUPAC recommendations as described for example in Markley et al., Pure and Appl. Chem. 70:117-142 (1998).

EXAMPLE X

5 **Use of Natural Log E-Value Ratios in Determining Pharmacofamily Membership Based on Sequence Models**

This example demonstrates identification of pharmacofamily members based on relative scores for E values of candidate members identified from searching a 10 database with a sequence model. The method is particularly useful for identifying members of a pharmacofamily in cases where differences in E values for members and non members is relatively small.

Polypeptides in pharmacofamily 1 were 15 structurally aligned with PRISM and a Hidden Markov Model was produced for the aligned polypeptides using HMMER 2.1 as described in Example VII. The training set for the first Hidden Markov Model includes all of the residues shown in Figure 11. The PDB sequence library was 20 searched with the first Hidden Markov Model as described in Example VII.

The search performed with the Hidden Markov Model derived from pharmacofamily 1 returned a set of polypeptides having E values in a range including values 25 less than and greater than 1 as shown in Table 15. In contrast to the results presented in Example VII for pharmacofamily 3, a large inflection was not observed in a plot of $-\ln(E)$ versus L as shown in Figure 12.

The following method was used to more clearly identify the demarcation between members and nonmembers of pharmacofamily 1. A ratio of the $-\ln(E)$ for the sequence compared against pharmacofamily 1 with the summed $-\ln(E)$ for pharmacofamilies 1 through 8 was calculated. This ratio is here referred to as XCorr (for cross correlation).

$$XCorr = \frac{\ln(E)}{\sum_{i=1}^N \ln(E_i)}, \text{ where } N \text{ is the total number of pharmacofamilies in the analysis.}$$

As shown in Figure 14, where the triangles represent the XCorr values (multiplied by 100 for purposes of expressing as a percentage), a significant 'break point' in XCorr values occurred at the same location in the sequence list as that identified by differential filtering (see Example VIII). In particular, the break point occurred where XCorr dropped from the neighborhood of 100% to the neighborhood of zero. All sequences above the break point (having higher $-\ln(E)$ values than those at the break point) are members of pharmacofamily 1 and all sequences below the break point (having $-\ln(E)$ values less than those at the break point) are not members of pharmacofamily 1.

In general, each sequence member of pharmacofamily 1 had an XCorr value near 100%, indicating that the probability that the sequence belongs to the specified pharmacofamily is much higher than the probability that it belongs to a different pharmacofamily. Sequences with an XCorr value close to

zero for a given pharmacofamily have a greater probability of belonging to another pharmacofamily.

Those sequences that are below the break point in Figure 14 but have XCorr values significantly greater than zero (for example, the 15th and 16th from the end and having XCorr values close to 100%) are likely members of an unrepresented pharmacofamily, outside of the group of N pharmacofamilies in question. If however, the set of considered pharmacofamilies is known to span the entire protein family space, then these sequences may be 'distal' pharmacofamily members with characteristics that are under-represented in the pharmacofamily model used.

The XCorr analysis was automated in a software application called Gene Family Profiler as follows. The protein sequences and Hidden Markov Model files described in Example VII were formatted in FASTA and HMMER 2.1 format, respectively, and read into Gene Family Profiler. Minor formatting flaws in the sequence file were automatically identified and corrected by the program. The sequences were searched by the Hidden Markov Models using the HMMER 2.1 program and E-values were calculated. Sequences having E-values at or below a predefined cutoff of 10 were compiled for further analysis (this cutoff E value can be altered by the user as necessary). For sequences having E-values that were above the cutoff, an XCorr value was calculated.

A summary of E values and XCorr values for each sequence was displayed as output from the program. As an example, the output indicated that sequence 1b61 is most likely a member of pharmacofamily 1 because it scored an

E-value from HMMER above the cutoff for only this pharmacofamily Hidden Markov Model and had an XCorr value of 1 for pharmacofamily 1. The sequence 1nda had E-values above the cutoff for both pharmacofamily 1 and 5 pharmacofamily 7. However, the 1nda sequence had Xcorr values of 1.0053 for pharmacofamily 7 and -0.0053 for pharmacofamily 1, respectively, indicating membership in pharmacofamily 7, rather than pharmacofamily 1.

The Gene Family Profiler software application 10 was further programed to carry out a secondary search for sequences that did not have a probability of belonging to any of the 8 pharmacofamilies represented by the Hidden Markov Models. If no significant similarities were found for a sequence to the pharmacofamilies in the primary 15 search with the Hidden Markov Model, the sequence was analyzed by the PSI-BLAST program (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)) against a library containing sequences of known members of all pharmacofamilies. Thus, the automated methods can be 20 used to find sequences in the family that are similar to a query sequence independent of pharmacofamily membership. Results of the secondary search can be used to further evaluate the similarity of the query sequence to the family as a whole.

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Table 3A: Pharmacofamily 1 subset

| Molecule # | pdb | type | RMSD from Family Avg. |
|------------|------|--------------------------------------------------------------|--------------------------------|
| 1 | 1A4I | Tetrahydrofolate Reductase (human) | 0.75 |
| 2 | 1AXE | Alcohol Dehydrogenase (horse) | 0.27 |
| 3 | 1DXY | D2-Hydroxyisocaproate Dehydrogenase (L. Casei) | 0.92 |
| 4 | 1LDN | L-Lactate Dehydrogenase (B. Stearothermophilus) | 0.41 |
| 5 | 1QR6 | Malic Enzyme (human) | 0.77 |
| 6 | 4MDH | Malate Dehydrogenase (pig) | 0.65 |
| 7 | 1AGN | Alcohol Dehydrogenase (human class IV sigma) | 0.63 |
| 8 | 1B3R | Adenosylhomocysteine (rat) | 0.93 |
| 9 | 1EMD | Malate Dehydrogenase (E. Coli) | 0.90 |
| 10 | 1PJC | L-Alanine (Phormidium Lapideum) | 0.79 |
| 11 | 1YKF | Alcohol Dehydrogenase (Thermoanaerobium Brockii) | 1.06 |
| 12 | 9IDB | Lactate Dehydrogenase (pig) | 0.36 |
| 13 | 1ARZ | Dihydrodipicolinate Reductase (E. Coli) | 0.81 |
| 14 | 1BMD | Malate Dehydrogenase (Thermus Flavis) | 0.68 |
| 15 | 1HYH | L2-Hydroxyisocaproate Dehydrogenase (Lactobacillus Confusus) | 0.57 |
| 16 | 1PSD | D3-Phosphoglycerate Dehydrogenase (E.Coli) | 0.78 |
| 17 | 2NAD | Formate Dehydrogenase (methylotrophic bacterium pseudomonas | 0.91 |

Table 3B: Polypeptide and Solvent Interactors (average coordinates)

| atom name | name | total | x | ox | y | oy | z | oz |
|-----------|------|-------|--------|------|-------|------|-------|------|
| A15 | ACC | 15 | -3.51 | 0.52 | -1.48 | 0.44 | -4.24 | 0.49 |
| A22 | ACC | 17 | 3.14 | 0.41 | -2.17 | 0.33 | -4.13 | 1.01 |
| A32 | ACC | 5 | 7.37 | 0.45 | 1.75 | 1.11 | -8.24 | 0.79 |
| A34 | ACC | 6 | 1.20 | 0.42 | 6.08 | 0.33 | -1.83 | 1.39 |
| A47 | ACC | 13 | -12.03 | 0.32 | -1.22 | 0.56 | -3.63 | 0.52 |
| A48 | ACC | 14 | -10.58 | 0.37 | -0.79 | 0.39 | -4.81 | 0.25 |
| A53 | ACC | 11 | -2.66 | 0.31 | -2.95 | 0.58 | -1.04 | 0.46 |
| A57 | ACC | 11 | 7.56 | 0.73 | -2.50 | 0.42 | -6.36 | 0.45 |
| A96 | ACC | 6 | 10.24 | 0.42 | 0.50 | 0.64 | -2.97 | 0.32 |
| A99 | ACC | 4 | 1.44 | 0.22 | 6.19 | 0.26 | -5.24 | 0.38 |
| D9 | DON | 17 | -7.70 | 0.67 | 2.30 | 0.43 | -6.27 | 0.29 |
| D10 | DON | 17 | -5.49 | 0.58 | 5.00 | 0.44 | -5.79 | 0.28 |
| D12 | DON | 17 | -3.06 | 0.53 | 4.22 | 0.42 | -7.05 | 0.38 |
| D34 | DON | 2 | 7.05 | 0.16 | 1.64 | 0.42 | -7.81 | 0.74 |
| D36 | DON | 4 | 1.28 | 0.39 | 6.13 | 0.37 | -1.01 | 0.70 |
| D53 | DON | 5 | -14.97 | 0.29 | 3.01 | 0.15 | -1.95 | 0.55 |
| D61 | DON | 11 | 2.46 | 0.64 | -2.82 | 0.54 | -0.35 | 0.58 |
| D84 | DON | 11 | 4.78 | 0.45 | 0.00 | 0.90 | -0.25 | 0.46 |
| D105 | DON | 7 | 10.22 | 0.38 | 0.54 | 0.59 | -3.10 | 0.45 |
| D148 | DON | 4 | -3.98 | 0.86 | 7.02 | 0.14 | -1.61 | 0.33 |
| W1 | WAT | 14 | -4.88 | 0.34 | 1.26 | 0.38 | -5.81 | 0.27 |
| W6 | WAT | 6 | -10.83 | 0.37 | 3.79 | 0.41 | -3.11 | 0.70 |
| W19 | WAT | 3 | -12.43 | 0.10 | 2.22 | 0.31 | -5.57 | 0.42 |

Table 3C: NAD(P) Conformer Model

| atom name | total | x | ox | y | oy | z | oz |
|-----------|-------|--------|------|-------|------|-------|------|
| PA | 17 | -5.47 | 0.22 | 3.43 | 0.30 | -1.84 | 0.27 |
| O2A | 17 | -5.82 | 0.31 | 4.60 | 0.37 | -2.38 | 0.65 |
| O1A | 17 | -5.72 | 0.50 | 3.38 | 0.60 | -0.59 | 0.64 |
| O5'A | 17 | -6.13 | 0.25 | 2.22 | 0.25 | -2.57 | 0.37 |
| C5'A | 17 | -6.23 | 0.13 | 0.92 | 0.22 | -2.20 | 0.23 |
| C4'A | 17 | -7.50 | 0.39 | 0.21 | 0.43 | -2.82 | 0.24 |
| O4'A | 17 | -7.46 | 0.19 | -1.07 | 0.14 | -2.48 | 0.34 |
| C3'A | 17 | -8.76 | 0.20 | 0.85 | 0.28 | -2.35 | 0.43 |
| O3'A | 17 | -9.62 | 0.37 | 1.13 | 0.33 | -3.41 | 0.67 |
| C2'A | 17 | -9.32 | 0.23 | -0.09 | 0.31 | -1.58 | 0.37 |
| O2'A | 17 | -10.69 | 0.36 | -0.06 | 0.51 | -1.72 | 0.54 |
| C1'A | 17 | -8.69 | 0.37 | -1.29 | 0.45 | -2.19 | 0.31 |
| N9A | 17 | -8.88 | 0.18 | -2.60 | 0.08 | -1.36 | 0.24 |
| C8A | 17 | -8.67 | 0.23 | -2.75 | 0.20 | -0.03 | 0.24 |
| N7A | 17 | -8.84 | 0.32 | -4.00 | 0.25 | 0.37 | 0.15 |
| C5A | 17 | -9.17 | 0.33 | -4.65 | 0.16 | -0.75 | 0.14 |
| C6A | 17 | -9.46 | 0.45 | -6.00 | 0.16 | -0.92 | 0.24 |
| N6A | 17 | -9.49 | 0.52 | -6.85 | 0.31 | 0.08 | 0.37 |
| N1A | 17 | -9.74 | 0.48 | -6.40 | 0.12 | -2.17 | 0.29 |
| C2A | 17 | -9.75 | 0.40 | -5.55 | 0.19 | -3.19 | 0.18 |
| N3A | 17 | -9.49 | 0.29 | -4.26 | 0.16 | -3.07 | 0.11 |
| C4A | 17 | -9.20 | 0.23 | -3.82 | 0.08 | -1.83 | 0.13 |
| O3 | 17 | -4.01 | 0.22 | 3.14 | 0.33 | -2.03 | 0.34 |
| PN | 17 | -2.81 | 0.17 | 3.31 | 0.22 | -2.96 | 0.33 |
| O1N | 17 | -2.32 | 0.49 | 4.39 | 0.63 | -2.89 | 0.71 |
| O2N | 17 | -3.16 | 0.47 | 3.27 | 0.61 | -4.13 | 0.54 |
| O5'N | 17 | -1.87 | 0.29 | 2.15 | 0.26 | -2.49 | 0.48 |
| C5'N | 17 | -1.92 | 0.27 | 0.87 | 0.27 | -2.66 | 0.46 |

| | | | | | | | |
|------|----|--------|------|-------|------|-------|------|
| C4'N | 17 | -0.83 | 0.19 | 0.02 | 0.24 | -2.14 | 0.36 |
| O4'N | 17 | 0.32 | 0.21 | 0.20 | 0.36 | -2.95 | 0.27 |
| C3'N | 17 | -0.36 | 0.23 | 0.40 | 0.28 | -0.74 | 0.32 |
| O3'N | 17 | -0.18 | 0.47 | -0.71 | 0.40 | 0.01 | 0.35 |
| C2'N | 17 | 0.91 | 0.23 | 1.05 | 0.40 | -0.94 | 0.21 |
| O2'N | 17 | 1.65 | 0.44 | 0.84 | 0.85 | 0.08 | 0.32 |
| C1'N | 17 | 1.45 | 0.18 | 0.41 | 0.23 | -2.17 | 0.22 |
| N1N | 17 | 2.44 | 0.15 | 1.17 | 0.24 | -2.89 | 0.19 |
| C2N | 17 | 3.61 | 0.20 | 0.61 | 0.24 | -3.24 | 0.16 |
| C3N | 17 | 4.53 | 0.22 | 1.30 | 0.35 | -3.97 | 0.23 |
| C7N | 17 | 5.81 | 0.29 | 0.71 | 0.58 | -4.39 | 0.38 |
| O7N | 17 | 6.57 | 0.47 | 1.16 | 0.94 | -4.83 | 0.51 |
| N7N | 17 | 6.03 | 0.44 | -0.27 | 0.96 | -4.27 | 0.71 |
| C4N | 17 | 4.30 | 0.34 | 2.55 | 0.41 | -4.33 | 0.47 |
| C5N | 17 | 3.12 | 0.39 | 3.09 | 0.48 | -3.96 | 0.64 |
| C6N | 17 | 2.19 | 0.27 | 2.41 | 0.44 | -3.24 | 0.51 |
| P2' | 2 | -11.69 | 0.02 | 1.32 | 0.36 | -1.90 | 0.73 |
| OP1 | 2 | -12.69 | 0.51 | 0.79 | 0.45 | -1.31 | 1.66 |
| OP2 | 2 | -12.01 | 0.86 | 1.94 | 0.08 | -3.01 | 0.74 |
| OP3 | 2 | -11.04 | 0.61 | 2.17 | 0.59 | -1.12 | 0.07 |

Table 3D: Polypeptide and Solvent Interactors

| Acceptors | atom name | residue mol. # | residue # | total | x | σx | y | σy | z | σz |
|-----------|-----------|----------------|-----------|--------|---|--------|---|--------|---|----|
| O | ALA 1 | 215 | | -4.41 | | -1.37 | | -4.378 | | |
| O | VAL 2 | 268 | | -3.415 | | -1.508 | | -4.259 | | |
| O | CYS 4 | 95 | | -3.525 | | -1.391 | | -4.201 | | |
| O | VAL 5 | 392 | | -4.035 | | -1.223 | | -4.42 | | |
| O | VAL 6 | 86 | | -2.622 | | -2.525 | | -3.463 | | |
| O | VAL 7 | 268 | | -3.739 | | -1.583 | | -4.801 | | |

| | | | | | |
|-------|----|-----|--------|---------|--------|
| THR | 8 | | -3.374 | -1.505 | -3.621 |
| SER | 9 | | -3.338 | -0.96 | -4.215 |
| ALA10 | | | -4.168 | -1.334 | -4.262 |
| ALA11 | | | -3.642 | -1.13 | -4.963 |
| THR12 | 97 | | -2.827 | -1.527 | -3.709 |
| PHE13 | 79 | | -3.279 | -1.095 | -4.527 |
| VAL14 | 86 | | -2.698 | -2.451 | -3.496 |
| THR15 | 96 | | -3.708 | -1.231 | -4.403 |
| ASN17 | | | -3.847 | -1.386 | -4.942 |
| ACC | 15 | 15 | -3.508 | 0.51867 | -1.481 |
| CYS | 1 | 236 | 3.015 | -2.169 | -3.644 |
| VAL | 2 | 292 | 3.319 | -2.239 | -3.966 |
| THR | 3 | 232 | 3.626 | -2.073 | -5.277 |
| ALA | 4 | 136 | 2.873 | -1.964 | -3.884 |
| LEU | 5 | 419 | 3.566 | -2.603 | -2.54 |
| VAL | 6 | 128 | 2.902 | -2.638 | -3.394 |
| VAL | 7 | 292 | 3.435 | -2.183 | -4.536 |
| ILE | 8 | 298 | 2.705 | -2.013 | -5.149 |
| ILE | 9 | 117 | 3.267 | -2.016 | -3.572 |
| VAL10 | | 266 | 3.531 | -1.908 | -3.445 |
| VAL11 | | 265 | 2.245 | -2.153 | -5.774 |
| VAL12 | | 138 | 3.423 | -2.49 | -3.658 |
| GLY13 | | 102 | 3.045 | -2.197 | -3.332 |
| VAL14 | | 128 | 2.473 | -2.343 | -3.403 |
| ILE15 | | 141 | 3.095 | -2.691 | -3.316 |
| ALA16 | | 238 | 3.132 | -1.372 | -5.812 |
| THR17 | | 282 | 3.668 | -1.893 | -5.571 |
| ACC | 22 | 17 | 3.1365 | 0.40729 | -2.173 |
| THR | 1 | | 6.933 | 1.937 | -8.332 |
| ALA | 3 | | 7.27 | 2.615 | -9.402 |
| ASN | 8 | | 7.341 | 0.057 | -7.801 |
| CYS11 | | | 8.12 | 2.802 | -8.368 |
| SER17 | | | 7.164 | 1.343 | -7.29 |

| | | | | | | | | | |
|------------|------------|-----------|-----------|---------------|----------------|---------------|-----------------|---------------|----------------|
| A32 | ACC | 32 | 5 | 7.3656 | 0.44907 | 1.7508 | 1.109256 | -8.239 | 0.78586 |
| SG | CYS | 2 | 46 | 1.759 | 6.095 | 6.095 | 6.095 | -1.597 | -1.597 |
| OG | SER | 6 | 240 | 1.154 | 5.714 | 5.714 | 5.714 | -0.415 | -0.415 |
| SG | CYS | 7 | 46 | 1.39 | 6.091 | 6.091 | 6.091 | -1.637 | -1.637 |
| OD1 | ASN | 8 | 190 | 1.47 | 6.205 | 6.205 | 6.205 | -3.174 | -3.174 |
| OG | SER | 9 | 222 | 0.831 | 6.625 | 6.625 | 6.625 | -0.409 | -0.409 |
| OG | SER10 | | 133 | 0.616 | 5.761 | 5.761 | 5.761 | -3.752 | -3.752 |
| A34 | ACC | 34 | 6 | 1.2033 | 0.42444 | 6.0818 | 0.331268 | -1.831 | 1.38661 |
| OD1 | ASP | 2 | 223 | -12.06 | -1.364 | -1.364 | -1.364 | -3.72 | -3.72 |
| OD1 | ASP | 3 | 175 | -12.31 | -1.116 | -1.116 | -1.116 | -2.892 | -2.892 |
| OD1 | ASP | 4 | 52 | -12.29 | -1.122 | -1.122 | -1.122 | -4.018 | -4.018 |
| OD2 | ASP | 6 | 41 | -12.14 | -1.461 | -1.461 | -1.461 | -3.317 | -3.317 |
| OD2 | ASP | 7 | 223 | -12.26 | 0.192 | 0.192 | 0.192 | -5.072 | -5.072 |
| OE1 | GLU | 8 | 242 | -12.17 | -0.604 | -0.604 | -0.604 | -3.687 | -3.687 |
| OD1 | ASP | 9 | 34 | -11.26 | -2.188 | -2.188 | -2.188 | -3.753 | -3.753 |
| OD2 | ASP10 | | 197 | -12.39 | -1.306 | -1.306 | -1.306 | -3.358 | -3.358 |
| OD1 | ASP12 | | 53 | -11.79 | -1.526 | -1.526 | -1.526 | -3.647 | -3.647 |
| CE1 | GLU14 | | 41 | -11.76 | -1.641 | -1.641 | -1.641 | -3.303 | -3.303 |
| OD1 | ASP15 | | 53 | -11.95 | -1.38 | -1.38 | -1.38 | -3.606 | -3.606 |
| OD1 | ASP16 | | 181 | -12.33 | -1.128 | -1.128 | -1.128 | -3.23 | -3.23 |
| OD1 | ASP17 | | 221 | -11.74 | -1.235 | -1.235 | -1.235 | -3.585 | -3.585 |
| A47 | ACC | 47 | 13 | -12.03 | 0.32497 | -1.221 | 0.556926 | -3.63 | 0.51984 |
| OD2 | ASP | 2 | 223 | -10.46 | -0.712 | -0.712 | -0.712 | -5.067 | -5.067 |
| OD2 | ASP | 3 | 175 | -10.78 | -0.582 | -0.582 | -0.582 | -4.327 | -4.327 |
| OD2 | ASP | 4 | 52 | -10.23 | -0.845 | -0.845 | -0.845 | -4.641 | -4.641 |
| OD1 | ASP | 6 | 41 | -10.8 | -0.87 | -0.87 | -0.87 | -4.98 | -4.98 |
| OD1 | ASP | 7 | 223 | -10.78 | -1.36 | -1.36 | -1.36 | -4.58 | -4.58 |
| OE2 | GLU | 8 | 242 | -10.46 | 0.103 | 0.103 | 0.103 | -4.803 | -4.803 |
| OD2 | ASP | 9 | 34 | -9.97 | -1.147 | -1.147 | -1.147 | -5.144 | -5.144 |
| OD1 | ASP10 | | 197 | -10.71 | -0.756 | -0.756 | -0.756 | -4.609 | -4.609 |
| OD2 | ASP12 | | 53 | -10.1 | -0.987 | -0.987 | -0.987 | -4.85 | -4.85 |
| OE1 | GLU13 | | 38 | -11.44 | -1.444 | -1.444 | -1.444 | -4.68 | -4.68 |
| OE2 | GLU14 | | 41 | -10.7 | -0.348 | -0.348 | -0.348 | -4.708 | -4.708 |

| | | | | | | | | | |
|------------|--|------------|-----------|-----------|---------------|----------------|---------------|-----------------|---------------|
| OD2 | | ASP15 | 53 | | -10.49 | | -0.813 | | -5.102 |
| OD2 | | ASP16 | 181 | | -10.87 | | -0.595 | | -4.761 |
| OD2 | | ASP17 | 221 | | -10.38 | | -0.678 | | -5.134 |
| A48 | | ACC | 48 | 14 | -10.58 | 0.37106 | -0.788 | 0.394449 | -4.813 |
| O | | ILE 2 | 269 | | -2.445 | | -2.256 | | -0.193 |
| O | | VAL 3 | 205 | | -2.446 | | -3.051 | | -1.43 |
| O | | ALA 4 | 96 | | -3.129 | | -3.442 | | -1.462 |
| OG | | SER 6 | 88 | | -2.227 | | -3.432 | | -0.657 |
| O | | ILE 7 | 269 | | -2.544 | | -2.277 | | -0.546 |
| O | | ALA 9 | 77 | | -2.936 | | -3.387 | | -1.405 |
| O | | VAL10 | 238 | | -2.653 | | -2.624 | | -0.587 |
| O | | ALA12 | 98 | | -3.101 | | -4.038 | | -1.238 |
| O | | THR13 | 80 | | -2.808 | | -2.299 | | -1.065 |
| O | | LEU15 | 97 | | -2.726 | | -2.902 | | -1.459 |
| O | | VAL16 | 211 | | -2.296 | | -2.734 | | -1.354 |
| A53 | | ACC | 53 | 11 | -2.665 | 0.30695 | -2.949 | 0.580767 | -1.036 |
| O | | ALA 2 | 317 | | 7.471 | | -2.554 | | -6.143 |
| OD2 | | ASP 3 | 258 | | 8.172 | | -2.402 | | -6.366 |
| OG | | SER 4 | 161 | | 7.049 | | -2.744 | | -6.487 |
| O | | LEU 6 | 154 | | 8.715 | | -2.807 | | -5.528 |
| O | | CYS 7 | 317 | | 7.229 | | -2.526 | | -6.12 |
| O | | VAL 9 | 146 | | 7.764 | | -1.709 | | -6.821 |
| OG | | SER12 | 163 | | 6.66 | | -2.956 | | -6.767 |
| O | | MET14 | 154 | | 8.194 | | -2.694 | | -5.797 |
| OG1 | | THR15 | 166 | | 6.339 | | -2.915 | | -6.856 |
| OD2 | | ASP16 | 264 | | 8.236 | | -1.758 | | -6.216 |
| OD1 | | ASP17 | 308 | | 7.288 | | -2.414 | | -6.878 |
| A57 | | ACC | 57 | 11 | 7.5561 | 0.73228 | -2.498 | 0.420521 | -6.362 |
| ND1 | | HIS 4 | 193 | | 10.626 | | 0.61 | | -3.116 |
| ND1 | | HIS 6 | 186 | | 10.014 | | -0.093 | | -2.576 |
| ND1 | | HIS 9 | 177 | | 10.504 | | 1.695 | | -3.436 |
| ND1 | | HIS12 | 195 | | 10.555 | | 0.375 | | -3.145 |
| ND1 | | HIS14 | 186 | | 9.53 | | 0.058 | | -2.803 |

| | | | | | |
|-----|-------|-----|--------|---------|----------|
| N | GLU 5 | 314 | -5.224 | 5.566 | -5.679 |
| N | GLN 6 | 14 | -6.138 | 5.075 | -5.705 |
| N | GLY 7 | 202 | -5.115 | 5.35 | -5.842 |
| N | ASP 8 | 222 | -4.822 | 4.792 | -5.908 |
| N | GLY 9 | 11 | -6.29 | 5.058 | -5.51 |
| N | VAL10 | 177 | -5.677 | 4.573 | -6.103 |
| N | PRO11 | 177 | -5.131 | 5.547 | -5.772 |
| N | ALA12 | 31 | -5.256 | 4.982 | -5.907 |
| N | ARG13 | 16 | -5.501 | 5.429 | -5.154 |
| N | GLN14 | 14 | -6.311 | 5.136 | -5.537 |
| N | ASN15 | 31 | -5.383 | 4.826 | -5.877 |
| N | HIS16 | 161 | -5.882 | 5.126 | -5.388 |
| N | ARG17 | 201 | -6 | 4.758 | -5.866 |
| D10 | DON | 10 | -5.492 | 0.57597 | 0.439163 |
| N | VAL 1 | 177 | -2.231 | 4.172 | -8.191 |
| N | VAL 2 | 203 | -2.521 | 4.333 | -7.106 |
| N | ILE 3 | 156 | -3.616 | 4.356 | -7.328 |
| N | VAL 4 | 31 | -2.539 | 3.702 | -7.072 |
| N | ALA 5 | 315 | -2.542 | 4.593 | -6.385 |
| N | ILE 6 | 15 | -3.471 | 4.432 | -7.048 |
| N | VAL 7 | 203 | -2.643 | 4.75 | -6.934 |
| N | VAL 8 | 223 | -2.523 | 3.344 | -6.862 |
| N | ILE 9 | 12 | -3.863 | 4.694 | -6.846 |
| N | VAL10 | 178 | -3.08 | 3.512 | -7.145 |
| N | VAL11 | 178 | -2.953 | 4.368 | -7.142 |
| N | VAL12 | 32 | -2.793 | 3.892 | -6.902 |
| N | MET13 | 17 | -3.251 | 4.443 | -6.48 |
| N | ILE14 | 15 | -3.826 | 4.526 | -7.009 |
| N | VAL15 | 32 | -2.951 | 3.934 | -7.082 |
| N | ILE16 | 162 | -3.722 | 4.618 | -7.096 |
| N | ILE17 | 202 | -3.556 | 4.064 | -7.229 |
| D12 | DON | 12 | -3.064 | 0.53062 | 4.2196 |
| OG1 | THR 1 | 279 | 6.933 | 1.937 | -8.332 |

| | | | | | | |
|-----|-------|-----|----|--------|---------|--------------------------------|
| OG | SER17 | 334 | | 7.164 | 1.343 | -7.29 |
| D34 | DON | 34 | 2 | 7.0485 | 0.16334 | 1.64 0.420021 -7.811 0.73681 |
| SG | CYS 2 | 46 | | 1.759 | 6.095 | -1.597 |
| OG | SER 6 | 240 | | 1.154 | 5.714 | -0.415 |
| SG | CYS 7 | 46 | | 1.39 | 6.091 | -1.637 |
| OG | SER 9 | 222 | | 0.831 | 6.625 | -0.409 |
| D36 | DON | 36 | 4 | 1.2835 | 0.39114 | 6.1313 0.374531 -1.015 0.6959 |
| ND2 | ASN 2 | 225 | | -14.56 | 3.056 | -1.923 |
| ND2 | ASN 7 | 225 | | -15.12 | 3.202 | -1.587 |
| ND2 | ASN10 | 199 | | -14.92 | 2.944 | -1.285 |
| N | ARG11 | 200 | | -15.34 | 3.078 | -2.669 |
| ND2 | ASN15 | 55 | | -14.92 | 2.794 | -2.271 |
| D53 | DON | 53 | 5 | -14.97 | 0.2886 | 3.0148 0.153705 -1.947 0.54651 |
| N | VAL 2 | 294 | | 2.334 | -2.69 | -0.397 |
| N | ASN 4 | 138 | | 2.277 | -2.379 | 0.029 |
| N | ASN 5 | 421 | | 2.644 | -2.578 | 0.583 |
| N | ASN 6 | 130 | | 2.063 | -2.785 | -0.349 |
| N | VAL 7 | 294 | | 2.742 | -3.152 | -1.066 |
| N | ASN 9 | 119 | | 2.504 | -2.09 | -0.346 |
| N | VAL10 | 268 | | 4.124 | -4.101 | -1.602 |
| N | ASN12 | 140 | | 2.522 | -2.522 | -0.359 |
| N | THR13 | 104 | | 2.237 | -3.331 | 0.05 |
| N | ASN14 | 130 | | 1.53 | -2.648 | -0.196 |
| N | ASN15 | 143 | | 2.106 | -2.7 | -0.15 |
| D61 | DON | 61 | 11 | 2.4621 | 0.64303 | -2.816 0.543046 -0.346 0.5762 |
| NH1 | ARG 3 | 234 | | 4.587 | -0.618 | 0.683 |
| ND2 | ASN 4 | 138 | | 5.58 | -1.025 | -0.579 |
| ND2 | ASN 5 | 421 | | 4.967 | -0.91 | -0.857 |
| ND2 | ASN 6 | 130 | | 4.796 | 0.498 | -0.376 |
| ND2 | ASN 9 | 119 | | 4.776 | 1.072 | -0.333 |
| ND2 | ASN12 | 140 | | 4.874 | 0.88 | -0.41 |
| ND2 | ASN14 | 130 | | 3.87 | 0.241 | -0.144 |
| ND2 | ASN15 | 143 | | 4.582 | 0.661 | -0.159 |

| atom name | residue mol. | residue # | total x | ox | y | sy | z | sz |
|-----------|--------------|-----------|---------|--------|---------|--------|----------|----------------|
| NH1 | ARG16 | 240 | 5.381 | -0.809 | -0.472 | | | |
| NH2 | ARG16 | 240 | 4.57 | 1.118 | 0.462 | | | |
| NH1 | ARG17 | 284 | 4.55 | -1.163 | -0.589 | | | |
| D84 | DON | 84 | 11 | 4.7757 | 0.4524 | -0.005 | 0.904651 | -0.252 0.45674 |
| ND1 | HIS 4 | 193 | 10.626 | 0.61 | -3.116 | | | |
| ND1 | HIS 6 | 186 | 10.014 | -0.093 | -2.576 | | | |
| ND1 | HIS 9 | 177 | 10.504 | 1.695 | -3.436 | | | |
| N | ASN10 | 299 | 10.126 | 0.746 | -3.889 | | | |
| ND1 | HIS12 | 195 | 10.555 | 0.375 | -3.145 | | | |
| ND1 | HIS14 | 186 | 9.53 | 0.058 | -2.803 | | | |
| ND1 | HIS15 | 198 | 10.182 | 0.378 | -2.754 | | | |
| D105 | DON | 105 | 7 | 10.22 | 0.38439 | 0.5384 | 0.587058 | -3.103 0.45095 |
| NE | ARG 9 | 80 | -3.463 | 6.961 | -1.445 | | | |
| NH1 | ARG12 | 101 | -3.963 | 7.113 | -1.977 | | | |
| NE | ARG13 | 16 | -3.284 | 7.146 | -1.239 | | | |
| NE2 | GLN14 | 14 | -5.2 | 6.85 | -1.788 | | | |
| D148 | DON | 148 | 4 | -3.978 | 0.86417 | 7.0175 | 0.137697 | -1.612 0.33227 |

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| | | | | | |
|-----|-------|-----|---------------|----------------|-----------------|
| O | HOH16 | 566 | -4.928 | 1.656 | -6.021 |
| O | HOH17 | 35 | -5.091 | 1.06 | -5.977 |
| W1 | WAT | 1 | -4.883 | 0.34302 | 1.255 |
| O | HOH 1 | 238 | -11.09 | 4.575 | 0.37899 |
| O | HOH 4 | 62 | -10.9 | 3.609 | -3.702 |
| O | HOH 6 | 71 | -10.22 | 3.569 | -3.539 |
| O | HOH10 | 92 | -11.17 | 3.592 | -2.078 |
| O | HOH15 | 395 | -10.54 | 3.897 | -2.43 |
| O | HOH17 | 199 | -11.04 | 3.484 | -3.702 |
| W6 | WAT | 6 | -10.83 | 0.37024 | 3.7877 |
| O | HOH 3 | 360 | -12.48 | 2.562 | -3.197 |
| O | HOH 5 | 495 | -12.31 | 1.96 | -5.14 |
| O | HOH17 | 439 | -12.49 | 2.145 | -5.591 |
| O | WAT | 19 | 3 | -12.43 | 0.09854 |
| W19 | | | | 2.2223 | 0.308361 |
| | | | | -5.57 | 0.41989 |

Table 4A: Pharmacofamily 2 Subset

| molecule # | pdb | type | rmsd from Family Avg. |
|------------|------|--------------------------------------------------------------|--------------------------------|
| 1 | 1CH6 | Glutamine Dehydrogenase (cow) | 0.58 |
| 2 | 1CER | Glyceraldehyde-3-phosphate D. (<i>Thermus aquaticus</i>) | 0.31 |
| 3 | 1GYP | Glyceraldehyde-3-phosphate D. (<i>Leishmania Mexicana</i>) | 0.34 |
| 4 | 2HDH | L3-hydroxyacyl CoA D. (human) | 0.33 |
| 5 | 1BXG | Phenylalanine D. (<i>Rhodococcus sp.</i>) | 0.59 |

Table 4B: Polypeptide and Solvent Interactors (average coordinates)

| Acceptors atom name | residue. mol. # | total | x | ox | y | oy | z | oz |
|------------------------|--------------------|-------|-------|------|-------|------|-------|------|
| A4 | ACC | 1 | 1.10 | - | -4.12 | - | 7.02 | - |
| A21 | ACC | 5 | -7.31 | 0.94 | 7.30 | 0.23 | 1.70 | 0.42 |
| A24 (D28) | ACC | 2 | -9.52 | 0.99 | 4.80 | 0.06 | -0.72 | 0.16 |
| A26 | ACC | 3 | -0.46 | 0.40 | 0.62 | 0.26 | 1.22 | 0.20 |
| A31 | ACC | 5 | 5.50 | 0.30 | 1.15 | 0.72 | 4.41 | 0.31 |
| A36 | ACC | 4 | 8.61 | 0.66 | -1.12 | 0.22 | 6.56 | 0.54 |
| A45 | ACC | 2 | -5.73 | 0.51 | 5.08 | 0.20 | -7.62 | 0.21 |
| A47 | ACC | 2 | -2.38 | 0.16 | 1.11 | 0.32 | 1.01 | 0.14 |
| A57 | ACC | 3 | 4.82 | 0.39 | 1.19 | 0.27 | 12.29 | 0.39 |
| A74 | ACC | 1 | 1.86 | - | -2.87 | - | 1.92 | - |
| A75 | ACC | 1 | 3.26 | - | -4.52 | - | 2.27 | - |
| A80 | ACC | 1 | 5.45 | - | -2.88 | - | 6.60 | - |

| Donors | atom name | residue. mol. # | total | x | σ_x | y | σ_y | z | σ_z |
|-----------|-----------|--------------------|--------|-------|------------|------|------------|------|------------|
| D21 | DON | 5 | -3.69 | 0.38 | 6.81 | 0.18 | 5.90 | 0.25 | |
| D22 | DON | 6 | -2.46 | 0.68 | 4.98 | 0.17 | 8.91 | 0.34 | |
| D24 | DON | 3 | 0.28 | 0.18 | 4.88 | 0.18 | 8.67 | 0.22 | |
| D27 | DON | 5 | -8.64 | 0.42 | 7.78 | 0.77 | -0.88 | 0.39 | |
| D28 (A24) | DON | 3 | -9.48 | 0.70 | 4.58 | 0.39 | -0.74 | 0.11 | |
| D37 | DON | 2 | 4.89 | 0.32 | -0.97 | 0.08 | 1.99 | 0.02 | |
| D38 | DON | 2 | 5.09 | 0.86 | -3.25 | 0.34 | 4.18 | 0.69 | |
| D84 | DON | 1 | -10.79 | - | 7.18 | - | 0.38 | - | |
| Water | atom name | residue. mol. # | total | x | σ_x | y | σ_y | z | σ_z |
| | W1 | WAT | 2 | -1.68 | 0.35 | 5.44 | 0.29 | 5.49 | 0.17 |

| Table 4C: NAD(P) Conformer Model | | | | | | | | | |
|----------------------------------|-----------|-------|-------|------------|------|------------|-------|------------|--|
| atom name | atom name | total | x | σ_x | y | σ_y | z | σ_z | |
| PA | PA | 5 | -4.24 | 0.19 | 1.80 | 0.11 | 6.48 | 0.23 | |
| O1A | | 5 | -5.08 | 0.52 | 0.75 | 0.25 | 6.07 | 0.45 | |
| O2A | | 5 | -4.62 | 0.23 | 2.55 | 0.14 | 7.71 | 0.23 | |
| O5'A | | 5 | -3.99 | 0.30 | 2.86 | 0.25 | 5.34 | 0.17 | |
| C5'A | | 5 | -4.32 | 0.41 | 2.73 | 0.18 | 4.00 | 0.21 | |
| C4'A | | 5 | -4.89 | 0.25 | 4.02 | 0.13 | 3.50 | 0.21 | |
| O4'A | | 5 | -4.66 | 0.06 | 4.05 | 0.14 | 2.08 | 0.25 | |
| C3'A | | 5 | -6.39 | 0.28 | 4.19 | 0.08 | 3.68 | 0.05 | |
| O3'A | | 5 | -6.70 | 0.35 | 5.46 | 0.12 | 4.28 | 0.08 | |
| C2'A | | 5 | -6.97 | 0.10 | 3.99 | 0.10 | 2.31 | 0.09 | |
| O2'A | | 5 | -8.13 | 0.10 | 4.75 | 0.15 | 2.08 | 0.23 | |
| C1'A | | 5 | -5.83 | 0.08 | 4.47 | 0.05 | 1.44 | 0.09 | |
| N9A | | 5 | -5.83 | 0.28 | 3.93 | 0.08 | 0.08 | 0.09 | |
| C8A | | 5 | -6.06 | 0.43 | 2.68 | 0.11 | -0.38 | 0.12 | |

| | C1'N | C2'N | C3'N | C4'N | C5'N | C6'N | C7'N | O1N | O2N | O3 | PN | N7A | C5A | C6A | C2A | N3A | C4A | O1N | O2N | O5'N | C5'N | C4'N | C4'N | C3'N | C2'N | O2'N | C1'N | C3'N | C7'N | O7N | C2N | C3N | C7N | O7N | C4N | C5N | C6N |
|---|-------|------|-------|------|-------|------|------|-------|------|-------|------|-------|------|------|-------|------|-------|------|-------|------|------|-------|------|-------|------|-------|------|------|-------|------|-------|------|-------|------|-----|-----|-----|
| 5 | -5.93 | 0.46 | 2.59 | 0.16 | -1.71 | 0.12 | 0.08 | -5.61 | 0.32 | 3.84 | 0.14 | -2.10 | 0.12 | 0.12 | -5.33 | 0.30 | 4.34 | 0.13 | -3.42 | 0.12 | 0.12 | -5.40 | 0.43 | 3.59 | 0.10 | -4.50 | 0.12 | 0.12 | -5.02 | 0.16 | 5.67 | 0.11 | -3.48 | 0.08 | | | |
| 5 | -5.02 | 0.16 | 5.67 | 0.11 | -3.48 | 0.08 | 0.07 | -4.98 | 0.15 | 6.46 | 0.10 | -2.39 | 0.12 | 0.07 | -5.23 | 0.19 | 6.03 | 0.05 | -1.15 | 0.15 | 0.07 | -5.53 | 0.23 | 4.70 | 0.09 | -1.02 | 0.07 | 0.07 | -5.53 | 0.23 | 4.70 | 0.09 | -1.02 | 0.07 | | | |
| 5 | -2.84 | 0.26 | 1.29 | 0.52 | 6.62 | 0.32 | 0.32 | -1.40 | 0.20 | 1.34 | 0.15 | 7.08 | 0.12 | 0.12 | -1.38 | 0.09 | 0.38 | 0.31 | 7.92 | 0.81 | 0.81 | -1.08 | 0.38 | 2.54 | 0.62 | 7.45 | 0.53 | 0.53 | -0.51 | 0.24 | 1.01 | 0.62 | 5.97 | 0.12 | | | |
| 5 | -0.51 | 0.24 | 1.01 | 0.62 | 5.97 | 0.12 | 0.12 | -0.17 | 0.26 | 1.53 | 0.19 | 4.90 | 0.36 | 0.36 | -0.17 | 0.22 | 0.97 | 0.17 | 4.29 | 0.20 | 0.20 | 1.07 | 0.22 | 0.97 | 0.17 | 4.29 | 0.20 | 0.20 | 2.15 | 0.28 | 1.09 | 0.07 | 5.24 | 0.14 | | | |
| 5 | 1.04 | 0.26 | -0.49 | 0.20 | 3.88 | 0.12 | 0.12 | 1.04 | 0.26 | -0.49 | 0.20 | 3.88 | 0.12 | 0.12 | 1.04 | 0.26 | -0.49 | 0.20 | 3.88 | 0.12 | 0.12 | 1.75 | 0.42 | -0.71 | 0.28 | 2.70 | 0.12 | 0.12 | 1.72 | 0.26 | -1.20 | 0.10 | 5.03 | 0.16 | | | |
| 5 | 2.24 | 0.33 | -2.42 | 0.17 | 4.63 | 0.40 | 0.40 | 2.24 | 0.33 | -2.42 | 0.17 | 4.63 | 0.40 | 0.40 | 2.24 | 0.33 | -2.42 | 0.17 | 4.63 | 0.40 | 0.40 | 2.76 | 0.26 | -0.18 | 0.11 | 5.44 | 0.12 | 0.12 | 3.11 | 0.26 | -0.28 | 0.02 | 6.85 | 0.14 | | | |
| 5 | 2.34 | 0.16 | -0.31 | 0.27 | 7.90 | 0.13 | 0.13 | 2.34 | 0.16 | -0.31 | 0.27 | 7.90 | 0.13 | 0.13 | 2.34 | 0.16 | -0.31 | 0.27 | 7.90 | 0.13 | 0.13 | 2.82 | 0.09 | -0.46 | 0.18 | 9.20 | 0.15 | 0.15 | 1.92 | 0.16 | -0.56 | 0.40 | 10.40 | 0.11 | | | |
| 5 | 2.01 | 0.59 | -0.69 | 0.67 | 11.28 | 0.54 | 0.54 | 2.01 | 0.59 | -0.69 | 0.67 | 11.28 | 0.54 | 0.54 | 2.01 | 0.59 | -0.69 | 0.67 | 11.28 | 0.54 | 0.54 | 0.66 | 0.05 | -0.71 | 1.04 | 10.09 | 0.19 | 0.19 | 4.19 | 0.10 | -0.48 | 0.22 | 9.46 | 0.21 | | | |
| 5 | 5.02 | 0.08 | -0.40 | 0.46 | 8.34 | 0.31 | 0.31 | 5.02 | 0.08 | -0.40 | 0.46 | 8.34 | 0.31 | 0.31 | 5.02 | 0.08 | -0.40 | 0.46 | 8.34 | 0.31 | 0.31 | 4.56 | 0.17 | -0.26 | 0.34 | 7.06 | 0.27 | 0.27 | 4.56 | 0.17 | -0.26 | 0.34 | 7.06 | 0.27 | | | |

Table 4D: Polypeptide and solvent Interactors

| Acceptors | atom name | residue mol. # | residue # | total | x | σ_x | y | σ_y | z | σ_z |
|------------|------------|----------------|-----------|---------------|----------------|---------------|-----------------|---------------|----------------|------------|
| OD1 | ASN 1 | 168 | | 1.095 | | -4.122 | | 7.015 | | |
| A4 | ACC | 4 | 1 | 1.095 | | -4.122 | | 7.015 | | |
| O | PHE 1 | 252 | | -5.191 | | 8.539 | | 6.797 | | |
| O | PHE 2 | 8 | | -5.255 | | 8.065 | | 6.21 | | |
| O | PHE 3 | 10 | | -4.805 | | 8.465 | | 5.853 | | |
| O | GLY 4 | 23 | | -4.854 | | 8.511 | | 7.292 | | |
| O | LEU 5 | 183 | | -5.255 | | 8.273 | | 6.6 | | |
| A14 | ACC | 14 | 5 | -5.072 | 0.22358 | 8.3706 | 0.199937 | 6.5504 | 0.55124 | |
| OE1 | GLU 1 | 275 | | -6.7 | | 7.256 | | 2.045 | | |
| OD1 | ASP 2 | 32 | | -8.197 | | 7.417 | | 1.98 | | |
| OD1 | ASP 3 | 38 | | -5.963 | | 7.483 | | 1.973 | | |
| OD1 | ASP 4 | 45 | | -7.792 | | 7.445 | | 1.259 | | |
| OD1 | ASP 5 | 205 | | -7.896 | | 6.916 | | 1.22 | | |
| A21 | ACC | 21 | 5 | -7.31 | 0.94194 | 7.3034 | 0.233204 | 1.6954 | 0.41735 | |
| OG | SER 1 | 276 | | -10.22 | | 4.761 | | -0.611 | | |
| OG1 | THR 5 | 206 | | -8.824 | | 4.845 | | -0.836 | | |
| A24 | ACC | 24 | 2 | -9.523 | 0.98783 | 4.803 | 0.059397 | -0.724 | 0.1591 | |
| O | ALA 1 | 326 | | -0.312 | | 0.409 | | 1.158 | | |
| O | ILE 4 | 108 | | -0.908 | | 0.539 | | 1.439 | | |
| O | ALA 5 | 239 | | -0.153 | | 0.904 | | 1.064 | | |
| A26 | ACC | 26 | 3 | -0.458 | 0.39802 | 0.6173 | 0.256629 | 1.2203 | 0.19512 | |
| O | GLY 1 | 347 | | 5.243 | | 2.256 | | 4.521 | | |
| O | THR 2 | 119 | | 5.496 | | 1.074 | | 4.297 | | |
| O | SER 3 | 134 | | 5.492 | | 0.484 | | 4.132 | | |
| O | ASN 4 | 135 | | 5.99 | | 0.551 | | 4.206 | | |
| O | ALA 5 | 260 | | 5.254 | | 1.362 | | 4.897 | | |
| A31 | ACC | 31 | 5 | 5.495 | 0.30275 | 1.1454 | 0.720452 | 4.4106 | 0.30869 | |
| OD1 | ASN 1 | 374 | | 9.186 | | -0.987 | | 5.966 | | |
| NE2 | HIS 4 | 158 | | 7.894 | | -1.364 | | 7.028 | | |

| | donor | atom name | residue mol. # | residue # | total | x | ox | y | oy | z | oz |
|------|-------|-----------|----------------|-----------|--------|---------|--------|----------|--------|---------|----|
| OD1 | ASN 5 | | | 288 | 8.756 | -0.995 | | | | 6.691 | |
| A36 | ACC | | 36 | 4 | 8.612 | 0.65793 | -1.115 | 0.215389 | 6.5617 | 0.54268 | |
| O | LYS 2 | | | 77 | -6.092 | 4.938 | | | -7.77 | | |
| O | GLN 3 | | | 91 | -5.369 | 5.217 | | | -7.467 | | |
| A45 | ACC | | 45 | 2 | -5.731 | 0.51124 | 5.0775 | 0.197283 | -7.619 | 0.21425 | |
| O | THR 2 | | | 96 | -2.488 | 1.334 | | | 0.905 | | |
| O | THR 3 | | | 111 | -2.265 | 0.887 | | | 1.109 | | |
| A47 | ACC | | 47 | 2 | -2.377 | 0.15768 | 1.1105 | 0.316077 | 1.007 | 0.14425 | |
| O | GLY 2 | | | 97 | -0.425 | -2.183 | | | -0.802 | | |
| O | GLY 3 | | | 112 | -0.663 | -2.629 | | | -0.591 | | |
| O | VAL 4 | | | 109 | -1.565 | -1.362 | | | -0.563 | | |
| A49 | ACC | | 49 | 3 | -0.884 | 0.60137 | -2.058 | 0.642683 | -0.652 | 0.13066 | |
| O | ASN 2 | | | 313 | 4.587 | 0.929 | | | 12.609 | | |
| O | ASN 3 | | | 335 | 5.271 | 1.175 | | | 12.408 | | |
| OGL1 | THR 5 | | | 153 | 4.596 | 1.474 | | | 11.859 | | |
| A57 | ACC | | 57 | 3 | 4.818 | 0.39234 | 1.1927 | 0.272929 | 12.292 | 0.38822 | |
| OE1 | GLU 4 | | | 110 | 1.86 | -2.87 | | | 1.915 | | |
| A74 | ACC | | 74 | 1 | 1.86 | -2.87 | | | 1.915 | | |
| OE2 | GLU 4 | | | 110 | 3.257 | -4.521 | | | 2.267 | | |
| A75 | ACC | | 75 | 1 | 3.257 | -4.521 | | | 2.267 | | |
| OG | SER 4 | | | 137 | 5.445 | -2.882 | | | 6.6 | | |
| A80 | ACC | | | 80 | 1 | 5.445 | -2.882 | | 6.6 | | |

| atom name | residue. | residue # | total | x | ox | y | oy | z | oz |
|-----------|----------|-----------|-------|--------|---------|--------|----------|--------|---------|
| | mol. # | | | | | | | | |
| O | HOH 4 | 888 | | -1.436 | | 5.238 | | | 5.606 |
| O | HOH 5 | 888 | | -1.931 | | 5.647 | | | 5.365 |
| W1 | WAT | 1 | 1 | -1.684 | 0.35002 | 5.4425 | 0.289207 | 5.4855 | 0.17041 |

Table 5A: Pharmacofamily 3 Subset

| Molecule # | pdb | type | RMSD from Family Avg. |
|------------|------|------------------------------------------------------|--------------------------------|
| 1 | 1A27 | 17 β -Hydroxysteroid Dehydrogenase (human) | 0.35 |
| 2 | 1AE1 | Tropinone Reductase | 0.33 |
| 3 | 1AHH | 7 α -Hydroxysteroid Dehydrogenase | 0.51 |
| 4 | 1BDB | Cis-Biphenyl-2,3-Dihydrodiol-2,3-Dehydrogenase | 0.28 |
| 5 | 1BSV | GDP-Fucose Synthase | 0.87 |
| 6 | 1CYD | Carbonyl Reductase | 0.26 |
| 7 | 1ENZ | Enoyl Acyl Carrier Protein Reductase | 0.66 |
| 8 | 1NAI | UDP-Galactose Epimerase | 0.45 |
| 9 | 1SEP | Sepiapterin Reductase | 0.43 |
| 10 | 1YBV | Trihydroxynaphthalene Reductase | 0.70 |
| 11 | 1HSD | 2 α -20 β -Hydroxysteroid Dehydrogenase | 0.55 |
| 12 | 1DIR | Dihydropteridine Reductase | 0.75 |

Table 5B: Polypeptide and solvent Interactors (average coordinates)

| Acceptors | atom name | Name | total | x | σx | y | σy | z | σz |
|-----------|-----------|------|--------|---------|------------|----------|------------|---------|------------|
| A5 (D5) | ACC | 4 | -9.243 | 0.6136 | -6.385 | 0.485759 | 7.5835 | 0.60521 | |
| A20 | ACC | 10 | -2.055 | 0.62558 | -12.31 | 0.344913 | 15.347 | 0.71676 | |
| A24 | ACC | 12 | -0.64 | 0.89267 | -1.809 | 0.373379 | 8.7658 | 0.66337 | |
| A32 | ACC | 12 | 2.8272 | 0.30273 | 5.1573 | 0.670541 | 10.018 | 0.502 | |
| A34 (D34) | ACC | 9 | 1.8439 | 0.50418 | 7.7642 | 0.274322 | 13.139 | 0.30794 | |
| A36 (D38) | ACC | 12 | -0.113 | 0.24453 | 4.7021 | 0.586493 | 13.952 | 0.24008 | |
| A38 | ACC | 11 | 1.2485 | 0.72569 | 9.7629 | 0.441462 | 9.482 | 0.48385 | |
| A40 | ACC | 10 | -2.496 | 0.41035 | 10.064 | 0.558296 | 8.9034 | 0.77733 | |
| A42 | ACC | 9 | -7.86 | 0.22197 | 8.1173 | 0.560664 | 9.1394 | 0.53745 | |
| A44 (D47) | ACC | 8 | -8.336 | 0.72492 | 4.1414 | 0.508189 | 9.0466 | 0.81437 | |

| Donors | | Name | Name | x | σ_x | y | σ_y | z | σ_z |
|-----------|------|------|--------|---------|------------|----------|------------|---------|------------|
| atom | name | | | | | | | | |
| D5 (A5) | DON | 6 | -9.892 | 1.12248 | -6.493 | 0.603878 | 7.9562 | 0.75319 | |
| D7 | DON | 2 | -9.66 | 0.00919 | -1.843 | 0.165463 | 8.0065 | 0.15061 | |
| D9 | DON | 12 | -6.057 | 0.41875 | 1.6692 | 0.293883 | 4.914 | 0.25367 | |
| D21 | DON | 10 | 0.0467 | 0.43511 | -11.62 | 0.342553 | 11.981 | 0.91633 | |
| D34 (A34) | DON | 9 | 1.8439 | 0.50418 | 7.7642 | 0.274322 | 13.139 | 0.30794 | |
| D38 (A36) | DON | 11 | -0.113 | 0.24453 | 4.7021 | 0.586493 | 13.952 | 0.24008 | |
| D40 | DON | 12 | 2.4988 | 0.36354 | 1.5627 | 0.445563 | 12.367 | 0.3007 | |
| D45 | DON | 10 | -5.476 | 0.54512 | 9.6232 | 0.478163 | 8.6938 | 0.41629 | |
| D47 (A44) | DON | 6 | -7.675 | 0.22275 | 3.8897 | 0.368935 | 9.5875 | 1.11949 | |

| Water | | Name | Name | x | σ_x | y | σ_y | z | σ_z |
|-------|------|------|--------|---------|------------|----------|------------|---------|------------|
| atom | name | | | | | | | | |
| W4 | WAT | 9 | -4.738 | 0.3561 | -1.037 | 0.298174 | 6.477 | 0.47268 | |
| W5 | WAT | 4 | 2.6995 | 0.66749 | -0.925 | 0.394841 | 9.7795 | 0.39679 | |
| W9 | WAT | 9 | 3.273 | 0.73202 | -1.012 | 0.573841 | 12.802 | 0.86657 | |
| W11 | WAT | 6 | -6.007 | 0.19132 | -1.829 | 0.200188 | 13.702 | 0.2296 | |

Table 5C: NAD(P) Conformer Model

| atom name | | x | σ_x | y | σ_y | z | σ_z | |
|-----------|--|----|------------|---------|------------|----------|------------|---------|
| PA | | 12 | -6.94 | 0.27682 | -0.359 | 0.12062 | 10.196 | 0.3132 |
| O1A | | 12 | -7.187 | 0.50362 | -0.724 | 0.311997 | 11.568 | 0.35149 |
| O2A | | 12 | -8.039 | 0.23033 | 0.0836 | 0.236246 | 9.4105 | 0.49965 |
| O5'A | | 12 | -6.324 | 0.33618 | -1.599 | 0.152174 | 9.5178 | 0.48615 |
| C5'A | | 12 | -5.31 | 0.27378 | -2.37 | 0.252109 | 9.8483 | 0.42032 |
| C4'A | | 12 | -5.39 | 0.23487 | -3.716 | 0.196458 | 9.4463 | 0.27041 |
| O4'A | | 12 | -4.443 | 0.17889 | -4.486 | 0.362347 | 10.152 | 0.45942 |
| C3'A | | 12 | -6.677 | 0.26263 | -4.369 | 0.172555 | 9.6349 | 0.38881 |
| O3'A | | 12 | -7.077 | 0.60241 | -4.969 | 0.317672 | 8.502 | 0.51095 |
| C2'A | | 12 | -6.427 | 0.2192 | -5.392 | 0.18758 | 10.719 | 0.34471 |

| | | | | | | | |
|------|----|--------|---------|--------|----------|--------|---------|
| O2'A | 12 | -7.207 | 0.43164 | -6.53 | 0.229629 | 10.538 | 0.52325 |
| C1'A | 12 | -4.996 | 0.2692 | -5.707 | 0.273621 | 10.514 | 0.28506 |
| N9A | 12 | -4.338 | 0.16157 | -6.335 | 0.231445 | 11.625 | 0.21234 |
| C8A | 12 | -4.321 | 0.18366 | -5.957 | 0.287413 | 12.906 | 0.25525 |
| N7A | 12 | -3.708 | 0.19062 | -6.853 | 0.38173 | 13.663 | 0.14123 |
| C5A | 12 | -3.345 | 0.167 | -7.802 | 0.336217 | 12.81 | 0.08303 |
| C6A | 12 | -2.685 | 0.29854 | -8.972 | 0.409416 | 13.085 | 0.20366 |
| N6A | 12 | -2.353 | 0.40839 | -9.302 | 0.557888 | 14.313 | 0.25603 |
| N1A | 12 | -2.439 | 0.38208 | -9.778 | 0.395034 | 12.051 | 0.30817 |
| C2A | 12 | -2.826 | 0.38939 | -9.443 | 0.393263 | 10.824 | 0.25264 |
| N3A | 12 | -3.468 | 0.30202 | -8.33 | 0.362823 | 10.533 | 0.10763 |
| C4A | 12 | -3.726 | 0.15519 | -7.514 | 0.288774 | 11.545 | 0.09427 |
| O3 | 12 | -5.803 | 0.33398 | 0.7197 | 0.195007 | 10.133 | 0.2437 |
| PN | 12 | -5.139 | 0.15801 | 1.6654 | 0.119922 | 9.0683 | 0.30355 |
| O1N | 12 | -5.513 | 0.30736 | 2.837 | 0.583522 | 9.2767 | 0.62893 |
| O2N | 12 | -5.465 | 0.24079 | 1.3618 | 0.579089 | 7.8578 | 0.57479 |
| O5'N | 12 | -3.623 | 0.17622 | 1.5297 | 0.454033 | 9.3583 | 0.46312 |
| C5'N | 12 | -2.693 | 0.23195 | 0.8583 | 0.262204 | 8.7345 | 0.42939 |
| C4'N | 12 | -1.318 | 0.21148 | 1.311 | 0.296942 | 9.1289 | 0.3066 |
| O4'N | 12 | -1.218 | 0.20704 | 2.7193 | 0.281646 | 8.9326 | 0.16566 |
| C3'N | 12 | -1.013 | 0.32386 | 1.0723 | 0.442515 | 10.567 | 0.32728 |
| O3'N | 12 | 0.2498 | 0.44917 | 0.5617 | 0.307845 | 10.743 | 0.48253 |
| C2'N | 12 | -1.071 | 0.433 | 2.4089 | 0.415664 | 11.195 | 0.2308 |
| O2'N | 12 | -0.264 | 0.66117 | 2.4258 | 0.295043 | 12.27 | 0.42485 |
| C1'N | 12 | -0.686 | 0.16367 | 3.3148 | 0.345237 | 10.094 | 0.21704 |
| N1N | 12 | -1.199 | 0.0741 | 4.663 | 0.296089 | 10.265 | 0.17649 |

| | C2N | C3N | C7N | O7N | N7N | C4N | C5N | C6N | P2' | OP1 | OP2 | OP3 |
|-----------|-----------|----------------|-----------|--------|---------|--------|----------|--------|---------|-----|-----|-------|
| Acceptors | atom name | residue mol. # | residue # | total | x | cx | y | cy | z | cz | | |
| O | GLY 1 | 0 | 9 | -4.643 | -4.27 | | | | | | | 6.043 |
| O | GLY 2 | 0 | 28 | -4.558 | -4.117 | | | | | | | 5.821 |
| O | GLY 3 | 0 | 18 | -4.048 | -4.273 | | | | | | | 6.088 |
| O | GLY 4 | 0 | 12 | -4.135 | -3.933 | | | | | | | 6.033 |
| O | GLY 5 | 0 | 10 | -4.432 | -4.169 | | | | | | | 5.555 |
| O | GLY 6 | 0 | 14 | -4.284 | -4.355 | | | | | | | 6.044 |
| O | GLY 7 | 0 | 14 | -6.249 | -5.065 | | | | | | | 6.52 |
| O | GLY 8 | 0 | 7 | -4.849 | -3.848 | | | | | | | 5.762 |
| O | GLY 9 | 0 | 15 | -4.591 | -3.878 | | | | | | | 5.357 |
| O | GLY10 | 0 | 36 | -4.346 | -4.384 | | | | | | | 5.754 |
| O | GLY11 | 0 | 13 | -5.058 | -4.026 | | | | | | | 6.159 |
| O | GLY12 | 0 | 13 | -5.622 | -4.826 | | | | | | | 5.87 |
| A1 | ACC | 1 | 1 | -4.735 | 0.64211 | -4.262 | 0.369162 | 5.9172 | 0.30204 | | | |
| OG | SER 1 | OG | 11 | -9.556 | -5.885 | | | | | | | 8.172 |
| OG | SER 2 | OG | 30 | -9.127 | -6.766 | | | | | | | 7.066 |
| OG | SER 8 | OG | 36 | -9.85 | -6.053 | | | | | | | 8.039 |

Table 5D: Polypeptide and Solvent Interactors

| | | | | | |
|--------------|-----------|--|---------------|----------------|----------------|
| SER 9 | | | -8.437 | -6.835 | 7.057 |
| OG | | | -9.243 | 0.6136 | -6.385 |
| A5 | 4 | | -1.811 | -12.31 | 14.284 |
| OD1 | | | -2.629 | -12.15 | 15.593 |
| OD1 | | | -1.583 | -12.75 | 16.533 |
| OD2 | | | -2.534 | -12.5 | 15.835 |
| OD2 | | | -2.109 | -11.85 | 15.924 |
| OD1 | | | -2.151 | -12.8 | 14.21 |
| OD1 | | | -2.841 | -11.82 | 15.085 |
| OD2 | | | -2.628 | -12.13 | 15.425 |
| OD1 | | | -1.218 | -12.17 | 15.492 |
| OD1 | | | -1.044 | -12.57 | 15.088 |
| OD1 | | | -2.055 | 0.62558 | -12.31 |
| ASP11 | 10 | | 0.344913 | 15.347 | 0.71676 |
| ACC | 20 | | -0.231 | -1.804 | 8.763 |
| O | | | -0.349 | -1.37 | 8.814 |
| O | | | 0.522 | -1.353 | 8.638 |
| O | | | 0.101 | -1.425 | 8.863 |
| O | | | -1.699 | -2.266 | 8.014 |
| O | | | -0.206 | -1.697 | 9.086 |
| O | | | -2.052 | -2.486 | 7.753 |
| O | | | -1.247 | -1.892 | 9.217 |
| O | | | -0.131 | -1.62 | 8.833 |
| O | | | 0.159 | -1.576 | 9.032 |
| O | | | -0.643 | -1.744 | 9.231 |
| O | | | -2.283 | -1.889 | 7.62 |
| O | | | -0.672 | 0.92482 | -1.76 |
| A24 | 12 | | 0.344669 | 8.6553 | 0.5546 |
| ACC | 24 | | 2.663 | 5.67 | 8.586 |
| O | | | 2.57 | 5.524 | 10.215 |
| O | | | 2.691 | 4.785 | 10.423 |
| O | | | 3.141 | 4.744 | 10.048 |
| O | | | 2.669 | 4.9 | 10.086 |
| O | | | 2.664 | 4.979 | 10.231 |
| O | | | 2.413 | 6.773 | 9.962 |

| | | | | | |
|----|------------|-----------|---------------|----------------|----------------|
| O | SER 8 | 123 | 3.033 | 5.584 | 9.704 |
| O | SER 9 | 157 | 2.652 | 5.344 | 10.012 |
| O | GLY10 | 163 | 3.026 | 4.753 | 10.51 |
| O | SER11 | 138 | 2.901 | 4.576 | 10.07 |
| O | GLY12 | 132 | 3.503 | 4.256 | 10.366 |
| O | ACC | 32 | 2.8272 | 0.30273 | 5.1573 |
| O | SER 1 | 142 | 1.908 | 7.501 | 12.689 |
| O | SER 2 | 158 | 1.217 | 8.135 | 13.294 |
| O | SER 3 | 146 | 1.984 | 7.724 | 13.283 |
| O | SER 4 | 142 | 2.278 | 7.462 | 12.615 |
| O | SER 5 | 107 | 1.06 | 7.551 | 13.088 |
| O | SER 8 | 124 | 2.726 | 8.12 | 13.565 |
| O | SER 9 | 158 | 1.901 | 8.072 | 13.351 |
| O | SER10 | 164 | 1.664 | 7.735 | 13.227 |
| O | SER11 | 139 | 1.857 | 7.578 | 13.136 |
| O | ACC | 34 | 9 | 1.8439 | 0.50418 |
| OH | TYR 1 | 155 | -0.171 | 5.291 | 14.251 |
| OH | TYR 2 | 171 | -0.291 | 4.635 | 13.936 |
| OH | TYR 3 | 159 | 0.016 | 5.509 | 14.332 |
| OH | TYR 4 | 155 | 0.03 | 4.468 | 13.891 |
| OH | TYR 5 | 136 | -0.098 | 3.379 | 13.966 |
| OH | TYR 6 | 149 | -0.376 | 4.379 | 13.778 |
| OH | TYR 8 | 149 | 0.166 | 4.681 | 13.768 |
| OH | TYR 9 | 171 | -0.28 | 4.756 | 13.633 |
| OH | TYR10 | 178 | -0.441 | 4.469 | 14.27 |
| OH | TYR11 | 152 | -0.176 | 4.772 | 13.685 |
| OH | TYR12 | 146 | 0.376 | 5.384 | 13.961 |
| O | ACC | 36 | 12 | -0.113 | 0.24453 |
| O | CYS 1 | 185 | 1.067 | 9.484 | 9.076 |
| O | PRO 2 | 201 | 0.576 | 10.012 | 9.398 |
| O | PRO 3 | 189 | 0.411 | 9.713 | 9.099 |
| O | SER 4 | 184 | 1.319 | 9.083 | 8.553 |
| O | PRO 5 | 163 | 2.198 | 10.158 | 9.311 |

| | | | | | |
|---|------------|-----------|---------------|----------------|----------------|
| O | PRO 6 | 179 | 0.756 | 9.916 | 10.316 |
| O | ALA 7 | 191 | 0.898 | 10.562 | 9.433 |
| O | TYR 8 | 177 | 1.702 | 10.131 | 9.844 |
| O | PRO10 | 208 | 1.679 | 9.684 | 9.536 |
| O | PRO11 | 182 | 0.511 | 9.318 | 9.88 |
| O | PRO12 | 178 | 2.617 | 9.331 | 9.856 |
| O | A38 | 38 | 1.2485 | 0.72569 | 9.7629 |
| O | GLY 1 | 186 | -2.149 | 9.494 | 8.888 |
| O | GLY 2 | 202 | -2.874 | 10.159 | 9.066 |
| O | GLY 3 | 190 | -2.748 | 9.972 | 8.954 |
| O | GLY 4 | 185 | -2.235 | 9.16 | 8.272 |
| O | THR 6 | 180 | -2.406 | 9.993 | 9.592 |
| O | GLY 7 | 192 | -2.617 | 10.505 | 8.651 |
| O | PHE 8 | 178 | -1.769 | 10.522 | 10.103 |
| O | GLY 9 | 200 | -2.438 | 9.522 | 8.495 |
| O | GLY11 | 183 | -2.476 | 10.303 | 9.636 |
| O | THR12 | 180 | -3.248 | 11.005 | 7.377 |
| O | A40 | 40 | -2.496 | 0.41035 | 10.064 |
| O | VAL 1 | 188 | -7.78 | 7.375 | 8.869 |
| O | ILE 2 | 204 | -8.015 | 7.969 | 8.848 |
| O | ILE 3 | 192 | -7.824 | 8.024 | 8.259 |
| O | ILE 4 | 187 | -8.021 | 7.996 | 9.727 |
| O | VAL 6 | 182 | -7.651 | 7.627 | 9.43 |
| O | ILE 7 | 194 | -7.928 | 8.273 | 9.726 |
| O | LEU 9 | 202 | -8.114 | 8.807 | 9.429 |
| O | ILE10 | 211 | -7.407 | 7.823 | 8.498 |
| O | THR11 | 185 | -7.996 | 9.162 | 9.469 |
| O | A42 | 42 | 9 | -7.86 | 0.22197 |
| O | OG1 | 190 | -7.639 | 3.969 | 9.24 |
| O | OG1 | 194 | -8.9 | 4.567 | 8.706 |
| O | SER 4 | 189 | -7.82 | 3.618 | 10.069 |
| O | OG1 | 184 | -7.838 | 4.124 | 9.427 |
| O | OG1 | 196 | -8.489 | 3.692 | 7.941 |

| | DON | 6 | -8.496 | 0.5257 | -3.866 | 0.346377 | 5.5828 | 0.41764 |
|-----|-------|----|--------|---------|---------|----------|----------|----------------|
| D6 | SER 1 | 12 | -9.666 | -1.96 | 8.113 | | | |
| OG | SER 4 | 15 | -9.653 | -1.726 | 7.9 | | | |
| OG | D7 | 7 | 2 | -9.66 | 0.00919 | -1.843 | 0.165463 | 8.0065 0.15061 |
| DON | GLY 1 | 13 | -8.789 | -0.1 | 5.426 | | | |
| N | GLY 2 | 32 | -9.284 | -0.05 | 5.677 | | | |
| N | GLY 3 | 22 | -8.761 | -0.722 | 5.167 | | | |
| N | GLY 4 | 16 | -8.685 | -0.121 | 5.731 | | | |
| N | MET 5 | 14 | -7.572 | 0.427 | 6.428 | | | |
| N | GLY 6 | 18 | -8.768 | -0.685 | 5.543 | | | |
| N | SER 7 | 20 | -9.948 | 1.364 | 5.27 | | | |
| N | TYR 8 | 11 | -8.49 | 0.13 | 6.189 | | | |
| N | GLY 9 | 19 | -9.129 | -0.325 | 6.034 | | | |
| N | GLY10 | 40 | -8.828 | -0.408 | 5.459 | | | |
| N | GLY11 | 17 | -8.878 | -0.198 | 5.546 | | | |
| N | ALA12 | 17 | -8.931 | -0.155 | 6.586 | | | |
| D8 | DON 8 | 12 | -8.839 | 0.5466 | -0.07 | 0.552142 | 5.7547 | 0.45545 |
| N | ILE 1 | 14 | -5.584 | 1.406 | 4.565 | | | |
| N | ILE 2 | 33 | -6.262 | 1.734 | 5.106 | | | |
| N | ILE 3 | 23 | -6.008 | 1.568 | 4.583 | | | |
| N | LEU 4 | 17 | -5.882 | 1.991 | 5.224 | | | |
| N | VAL 5 | 15 | -5.284 | 1.794 | 5.226 | | | |
| N | ILE 6 | 19 | -5.843 | 1.286 | 4.804 | | | |
| N | ILE 7 | 21 | -6.436 | 2.018 | 4.734 | | | |
| N | ILE 8 | 12 | -6.417 | 2.039 | 4.837 | | | |
| N | PHE 9 | 20 | -6.214 | 1.631 | 5.229 | | | |
| N | ILE10 | 41 | -5.852 | 1.601 | 5.016 | | | |
| N | LEU11 | 18 | -6.037 | 1.845 | 5.008 | | | |
| N | LEU12 | 18 | -6.861 | 1.117 | 4.636 | | | |
| D9 | DON 9 | 12 | -6.057 | 0.41875 | 1.6692 | 0.293883 | 4.914 | 0.25367 |
| N | LEU 1 | 36 | -4.861 | -11.14 | 5.491 | | | |
| N | SER 2 | 52 | -5.654 | -10.93 | 6.923 | | | |
| N | ASP 3 | 42 | -4.048 | -10.76 | 6.515 | | | |

| | | | | | |
|-----|------------|-----------|---------------|----------------|----------------|
| N | ASP 4 | 36 | -3.888 | -11 | 6.574 |
| N | THR 6 | 38 | -3.943 | -10.92 | 6.379 |
| N | PHE 7 | 41 | -6.508 | -10.95 | 7.546 |
| N | ALA 9 | 42 | -4.253 | -10.74 | 6.218 |
| N | TYR10 | 60 | -4.488 | -11.11 | 5.821 |
| N | ASP11 | 37 | -4.555 | -10.8 | 6.546 |
| N | ASP12 | 37 | -5.596 | -11.16 | 7.002 |
| D11 | DON | 11 | -4.779 | 0.8737 | -10.95 |
| N | VAL 1 | 66 | 0.188 | -11.57 | 12.02 |
| N | LEU 2 | 79 | -0.75 | -11.93 | 12.873 |
| N | ILE 3 | 69 | 0.555 | -10.96 | 12.368 |
| N | VAL 4 | 60 | 0.173 | -11.26 | 12.105 |
| N | LEU 6 | 61 | -0.617 | -11.88 | 13.014 |
| N | VAL 7 | 65 | -0.2 | -12.11 | 11.698 |
| N | ILE 8 | 59 | 0.203 | -11.54 | 11.611 |
| N | VALL0 | 88 | 0.182 | -11.52 | 12.416 |
| N | VALL1 | 61 | 0.252 | -11.53 | 11.99 |
| OH | TYR12 | 12 | 0.481 | -11.87 | 9.718 |
| D21 | DON | 21 | 0.0467 | 0.43511 | -11.62 |
| OG | SER 1 | 142 | 1.908 | 7.501 | 12.689 |
| OG | SER 2 | 158 | 1.217 | 8.135 | 13.294 |
| OG | SER 3 | 146 | 1.984 | 7.724 | 13.283 |
| OG | SER 4 | 142 | 2.278 | 7.462 | 12.615 |
| OG | SER 5 | 107 | 1.06 | 7.551 | 13.088 |
| OG | SER 8 | 124 | 2.726 | 8.12 | 13.565 |
| OG | SER 9 | 158 | 1.901 | 8.072 | 13.351 |
| OG | SER10 | 164 | 1.664 | 7.735 | 13.227 |
| OG | SER11 | 139 | 1.857 | 7.578 | 13.136 |
| D34 | DON | 34 | 9 | 1.8439 | 0.50418 |
| OH | TYR 1 | 155 | -0.171 | 5.291 | 14.251 |
| OH | TYR 2 | 171 | -0.291 | 4.635 | 13.936 |
| OH | TYR 3 | 159 | 0.016 | 5.509 | 14.332 |
| OH | TYR 4 | 155 | 0.03 | 4.468 | 13.891 |

| | | | | | |
|-------|----|-----|--------|---------|--------|
| TYR | 5 | 136 | -0.098 | 3.379 | 13.966 |
| TYR | 6 | 149 | -0.376 | 4.379 | 13.778 |
| TYR | 8 | 149 | 0.166 | 4.681 | 13.768 |
| TYR | 9 | 171 | -0.28 | 4.756 | 13.633 |
| TYR10 | | 178 | -0.441 | 4.469 | 14.27 |
| TYR11 | | 152 | -0.176 | 4.772 | 13.685 |
| TYR12 | | 146 | 0.376 | 5.384 | 13.961 |
| DON | 38 | 11 | -0.113 | 0.24453 | 4.7021 |
| LYS | 1 | 159 | 2.273 | 1.347 | 12.922 |
| LYS | 2 | 175 | 2.774 | 1.885 | 12.501 |
| LYS | 3 | 163 | 2.831 | 1.966 | 12.606 |
| LYS | 4 | 159 | 2.945 | 1.926 | 11.968 |
| LYS | 5 | 140 | 2.494 | 0.716 | 12.288 |
| LYS | 6 | 153 | 2.639 | 1.609 | 12.544 |
| LYS | 7 | 165 | 1.913 | 2.31 | 11.938 |
| LYS | 8 | 153 | 2.821 | 1.471 | 12.018 |
| LYS | 9 | 175 | 2.663 | 1.484 | 12.193 |
| LYS10 | | 182 | 2.338 | 1.274 | 12.644 |
| LYS11 | | 156 | 2.502 | 1.768 | 12.367 |
| LYS12 | | 150 | 1.793 | 0.996 | 12.411 |
| DON | 40 | 12 | 2.4988 | 0.36354 | 1.5627 |
| VAL | 1 | 188 | -5.575 | 9.076 | 8.69 |
| ILE | 2 | 204 | -5.985 | 9.861 | 8.611 |
| ILE | 3 | 192 | -5.491 | 9.652 | 7.982 |
| ILE | 4 | 187 | -5.774 | 9.173 | 8.669 |
| VAL | 6 | 182 | -5.726 | 9.411 | 9.22 |
| ILE | 7 | 194 | -5.844 | 10.081 | 9.195 |
| LEU | 9 | 202 | -5.489 | 9.563 | 8.577 |
| ILE10 | | 211 | -5.165 | 9.506 | 8.351 |
| THR11 | | 185 | -5.643 | 10.664 | 9.242 |
| LEU12 | | 181 | -4.064 | 9.245 | 8.401 |
| DON | 45 | 10 | -5.476 | 0.54512 | 9.6232 |
| THR1 | | 190 | -7.639 | 3.969 | 9.24 |

cont.

| | | | | | |
|-----|-------|-----|----------|----------------|-------------------------|
| OG | SER 4 | 189 | -7.82 | 3.618 | 10.069 |
| OG1 | THR 6 | 184 | -7.838 | 4.124 | 9.427 |
| NZ | LYS 8 | 84 | -7.399 | 3.308 | 11.527 |
| ND2 | ASN 9 | 204 | -7.429 | 3.984 | 8.246 |
| OG1 | THR10 | 213 | -7.925 | 4.335 | 9.016 |
| D47 | DON | 47 | 6 -7.675 | 0.22275 3.8897 | 0.368935 9.5875 1.11949 |

| Water | atom name | residue mol. # | residue # | total | x | σx | y | σy | z | σz |
|-------|-----------|----------------|-----------|--------|---------|--------|----------|--------|---------|----|
| | O | HOH 1 | 525 | -4.833 | | | -1.135 | | 6.451 | |
| | O | HOH 2 | 46 | -5.297 | | | -1.061 | | 6.752 | |
| | O | HOH 3 | 3 | -4.845 | | | -1.187 | | 6.502 | |
| | O | HOH 4 | 516 | -4.351 | | | -0.821 | | 6.859 | |
| | O | HOH 5 | 437 | -4.101 | | | -1.147 | | 6.704 | |
| | O | HOH 6 | 10 | -4.524 | | | -1.331 | | 6.783 | |
| | O | HOH 7 | 309 | -4.955 | | | -0.333 | | 5.377 | |
| | O | HOH 8 | 2 | -4.854 | | | -1.09 | | 6.112 | |
| | O | HOH 9 | 12 | -4.878 | | | -1.224 | | 6.753 | |
| | W4 | WAT 4 | 9 | -4.738 | 0.3561 | -1.037 | 0.298174 | 6.477 | 0.47268 | |
| | O | HOH 1 | 536 | 3.343 | | | -0.704 | | 9.644 | |
| | O | HOH 5 | 429 | 1.797 | | | -0.842 | | 9.926 | |
| | O | HOH 6 | 327 | 3.022 | | | -1.504 | | 10.239 | |
| | O | HOH 7 | 293 | 2.636 | | | -0.648 | | 9.309 | |
| | W5 | WAT 5 | 4 | 2.6995 | 0.66749 | -0.925 | 0.394841 | 9.7795 | 0.39679 | |
| | O | HOH 1 | 556 | 2.764 | | | -1.43 | | 12.516 | |
| | O | HOH 2 | 24 | 3.482 | | | -0.937 | | 11.868 | |
| | O | HOH 3 | 72 | 4.908 | | | -0.703 | | 11.31 | |
| | O | HOH 4 | 531 | 3.597 | | | -0.619 | | 12.808 | |
| | O | HOH 5 | 433 | 2.747 | | | -2.319 | | 13.306 | |
| | O | HOH 6 | 24 | 3.505 | | | -1.086 | | 12.854 | |
| | O | HOH 7 | 292 | 2.421 | | | -0.63 | | 12.788 | |
| | O | HOH 8 | 125 | 2.922 | | | -0.954 | | 13.552 | |

Table 6A: Pharmacofamily 4 Subset

| molecule # | pdb | type | rmsd from family avg. |
|------------|------|------------------------------|--------------------------------|
| 1 | 2CAH | catalyse (Proteus Mirabilis) | 0.18 |
| 2 | 8CAT | catalyse (cow) | 0.18 |

Table 6B: Polypeptide and Solvent Interactors (average coordinates)

| Acceptors | | total | x | σx | y | σy | z | σz |
|-----------|--------------------|-------|--------|---------|--------|----------|--------|---------|
| atom name | residue. mol. # | | | | | | | |
| A3 (D4) | ACC | 2 | -1.117 | 0.36133 | -3.964 | 0.13435 | -3.882 | 0.27082 |
| A6 (D7) | ACC | 2 | -10.03 | 0.10889 | -5.617 | 0.029698 | 1.223 | 0.1895 |
| A17 | ACC | 2 | 5.454 | 0.08697 | 2.473 | 0.195161 | -0.056 | 0.58973 |
| A19 (D30) | ACC | 2 | 3.405 | 0.48366 | 1.421 | 0.065761 | 4.934 | 0.05586 |
| A21 | ACC | 2 | 1.11 | 0.65478 | -7.271 | 0.181726 | -2.784 | 0.39527 |
| A35 | ACC | 2 | 3.372 | -7.545 | | | 0.205 | |

| Donors | | total | x | σx | y | σy | z | σz |
|-----------|--------------------|-------|--------|---------|--------|----------|--------|---------|
| atom name | residue. mol. # | | | | | | | |
| D4 (A3) | DON | 2 | -1.117 | 0.36133 | -3.964 | 0.13435 | -3.882 | 0.27082 |
| D7 (A6) | DON | 2 | -10.03 | 0.10889 | -5.617 | 0.029698 | 1.223 | 0.1895 |
| D10 | DON | 2 | -6.918 | 0.49215 | -1.253 | 0.286378 | 7 | 0.28284 |
| D11 | DON | 2 | -6.419 | 0.19163 | 0.023 | 0.147078 | 5.184 | 0.18173 |
| D14 | DON | 2 | -6.153 | 3.824 | | | 6.584 | |
| D21 | DON | 2 | -2.402 | 4.522 | | | 6.578 | |
| D22 | DON | 2 | -2.704 | 0.0997 | 4.738 | 0.703571 | 9.015 | 0.19658 |
| D26 | DON | 2 | 4.609 | 0.02758 | 2.264 | 0.350018 | -2.894 | 0.51831 |
| D30 (A19) | DON | 2 | 3.405 | 0.48366 | 1.421 | 0.065761 | 4.934 | 0.05586 |

D42

DON

D42

| Waters | atom name | residue mol. # | | total | x | σ_x | y | σ_y | z | σ_z |
|--------|-----------|----------------|--|-------|-------|------------|--------|------------|--------|------------|
| | W1 | WAT | | 2 | 2.756 | | 3.789 | | -1.727 | |
| | W3 | WAT | | 2 | 7.572 | | -1.978 | | 4.115 | |

Table 6C: NAD(P) Conformer Model

| atom name | number | x | σ_x | y | σ_y | z | σ_z |
|-----------|--------|-------|------------|-------|------------|------|------------|
| PA | 2 | 2.91 | 0.04 | -2.21 | 0.03 | 5.65 | 0.05 |
| O1A | 2 | 2.72 | 0.06 | -3.30 | 0.15 | 6.64 | 0.05 |
| O2A | 2 | 3.84 | 0.02 | -1.14 | 0.13 | 6.03 | 0.21 |
| O5'A | 2 | 1.43 | 0.11 | -1.58 | 0.12 | 5.49 | 0.10 |
| C5'A | 2 | 0.37 | 0.04 | -2.46 | 0.22 | 4.99 | 0.04 |
| C4'A | 2 | -0.65 | 0.05 | -1.65 | 0.13 | 4.29 | 0.00 |
| O4'A | 2 | -1.84 | 0.18 | -2.41 | 0.04 | 4.08 | 0.03 |
| C3'A | 2 | -1.09 | 0.10 | -0.66 | 0.26 | 5.21 | 0.33 |
| O3'A | 2 | -0.77 | 0.41 | 0.64 | 0.09 | 5.13 | 0.06 |
| C2'A | 2 | -2.37 | 0.16 | -1.05 | 0.21 | 5.80 | 0.03 |
| O2'A | 2 | -3.24 | 0.42 | 0.04 | 0.54 | 6.17 | 0.19 |
| C1'A | 2 | -3.00 | 0.12 | -1.63 | 0.23 | 4.60 | 0.08 |
| N9A | 2 | -4.14 | 0.04 | -2.49 | 0.13 | 4.54 | 0.09 |
| C8A | 2 | -4.58 | 0.08 | -3.42 | 0.00 | 5.41 | 0.04 |
| N7A | 2 | -5.62 | 0.12 | -4.11 | 0.07 | 5.01 | 0.00 |
| C5A | 2 | -5.86 | 0.04 | -3.62 | 0.02 | 3.74 | 0.06 |
| C6A | 2 | -6.85 | 0.05 | -3.94 | 0.05 | 2.77 | 0.07 |
| N6A | 2 | -7.79 | 0.12 | -4.87 | 0.11 | 2.95 | 0.01 |
| N1A | 2 | -6.82 | 0.06 | -3.25 | 0.04 | 1.61 | 0.11 |
| C2A | 2 | -5.88 | 0.13 | -2.29 | 0.16 | 1.45 | 0.15 |
| N3A | 2 | -4.93 | 0.16 | -1.91 | 0.18 | 2.28 | 0.15 |
| C4A | 2 | -4.98 | 0.06 | -2.62 | 0.08 | 3.43 | 0.10 |
| O3 | 2 | 3.16 | 0.09 | -2.77 | 0.20 | 4.19 | 0.05 |

Table 6D: Polypeptide and Solvent Interactors

| Acceptors | atom name | residue mol. # | residue # | total | x | ox | y | oy | z | sz |
|-----------|-----------|----------------|-----------|-------|-------|-------|-------|-------|-------|----|
| | NE2 | HIS 1 | 173 | | -1.37 | | -4.06 | | -3.69 | |
| | NE2 | HIS 2 | 193 | | -0.86 | | -3.87 | | -4.07 | |
| | A3 | ACC 3 | 2 | -1.12 | 0.36 | -3.96 | 0.13 | -3.88 | 0.27 | |

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| | | | | | | |
|------------|------------|-----------|----------|---------------|--------------|--------------|
| OG | SER | 1 | 180 | -10.10 | -5.60 | 1.09 |
| OG | SER | 2 | 200 | -9.95 | -5.64 | 1.36 |
| A6 | ACC | 6 | 2 | -10.03 | 0.11 | -5.62 |
| O | TRP | 1 | 282 | 5.52 | 2.34 | -0.47 |
| O | TRP | 2 | 302 | 5.39 | 2.61 | 0.36 |
| A17 | ACC | 17 | 2 | 5.45 | 0.09 | 2.47 |
| ND1 | HIS | 1 | 284 | 3.06 | 1.47 | 4.97 |
| ND1 | HIS | 2 | 304 | 3.75 | 1.38 | 4.89 |
| A19 | ACC | 19 | 2 | 3.41 | 0.48 | 1.42 |
| O | GLN | 1 | 421 | 0.65 | -7.40 | -2.50 |
| O | GLN | 2 | 441 | 1.57 | -7.14 | -3.06 |
| A21 | ACC | 21 | 2 | 1.11 | 0.65 | -7.27 |
| OG1 | THR | 2 | 444 | 3.37 | -7.55 | -2.78 |
| A35 | ACC | 35 | 2 | 3.37 | -7.55 | 0.21 |

Donors
atom name

| | | | | | | |
|-----|-------|-----|---|--------------|-------------|-------------|
| NH1 | ARG 1 | 216 | | -2.40 | 4.52 | 6.58 |
| D21 | DON | 21 | 2 | -2.40 | 4.52 | 6.58 |
| NH2 | ARG 1 | 216 | | -2.78 | 4.24 | 8.88 |
| NZ | LYS 2 | 236 | | -2.63 | 5.24 | 9.15 |
| D22 | DON | 22 | 2 | -2.70 | 0.10 | 4.74 |
| N | TRP 1 | 282 | | 4.59 | 2.02 | -3.26 |
| N | TRP 2 | 302 | | 4.63 | 2.51 | -2.53 |
| D26 | DON | 26 | 2 | 4.61 | 0.03 | 2.26 |
| ND1 | HIS 1 | 284 | | 3.06 | 1.47 | 4.97 |
| ND1 | HIS 2 | 304 | | 3.75 | 1.38 | 4.89 |
| D30 | DON | 30 | 2 | 3.41 | 0.48 | 1.42 |
| NE2 | GLN 2 | 281 | | 3.91 | 6.03 | 0.45 |
| D42 | DON | 42 | 2 | 3.91 | 6.03 | 0.45 |

Waters
atom name

| residue. mol. # | residue # | total | x | σx | y | σy | z | σz |
|--------------------|-----------|-------|---|-------------|--------------|------------|-------------|------------|
| O | HOH 1 | 10 | | 2.76 | 3.79 | | -1.73 | |
| W1 | WAT | 1 | 2 | 2.76 | 3.79 | | -1.73 | |
| O | HOH 1 | 12 | | 7.57 | -1.98 | | 4.12 | |
| W3 | WAT | 3 | 2 | 7.57 | -1.98 | | 4.12 | |

Table 7A: Pharmacofamily 5 subset

| Molecule # | pdb | type | RMSD from Family | Avg. |
|------------|------|-------------------------------------------------------|------------------------|------|
| 1 | 1A80 | 2,5-Diketo-D-Gluconic Acid Reductase (Corynebacterium | 0.21 | |
| 2 | 1AFS | 3-a-Hydroxysteroid Dehydrogenase (rat) | 0.66 | |
| 3 | 1FRB | Aldo-Keto Reductase (mouse) | 0.55 | |
| 4 | 1ADS | Aldose Reductase (human) | 0.55 | |
| 5 | 1AH0 | Aldose Reductase (pig) | 0.56 | |

Table 7B: Polypeptide and Solvent Interactors (average coordinates)

| Acceptors | atom name | residue. mol. # | total | x | σx | y | σy | z | σz |
|-----------|-----------|--------------------|-------|------|-------|------|--------|------|----|
| A3 | ACC | 5 | -0.31 | 0.38 | 8.08 | 0.84 | -3.93 | 0.51 | |
| A5 | ACC | 5 | -7.54 | 0.31 | 10.00 | 0.16 | 0.36 | 0.24 | |
| A8 (D6) | ACC | 5 | -3.86 | 0.33 | 10.11 | 0.12 | 2.13 | 0.21 | |
| A11 (D11) | ACC | 5 | -3.42 | 0.36 | 10.75 | 0.31 | 6.12 | 0.36 | |
| A14 (D15) | ACC | 5 | -7.65 | 0.42 | 8.35 | 0.28 | 7.93 | 0.19 | |
| A18 | ACC | 5 | -8.07 | 0.25 | 7.90 | 0.12 | 3.55 | 0.09 | |
| A32 (D35) | ACC | 5 | -3.37 | 0.49 | 3.38 | 0.29 | -11.88 | 0.27 | |
| A37 | ACC | 5 | -6.70 | 0.49 | -3.63 | 0.36 | -15.32 | 0.27 | |
| A38 | ACC | 5 | -7.25 | 0.30 | -4.35 | 0.17 | -13.39 | 0.20 | |
| A40 | ACC | 4 | -8.26 | 0.22 | -0.78 | 0.09 | -10.85 | 0.30 | |
| A42 (D21) | ACC | 4 | -4.11 | 0.29 | 3.97 | 0.06 | 7.45 | 0.05 | |
| A43 (D49) | ACC | 4 | -3.07 | 0.46 | 1.67 | 0.40 | 1.87 | 0.38 | |
| A55 (D65) | ACC | 3 | 0.11 | 0.37 | 1.66 | 0.18 | -0.35 | 0.22 | |
| A58 | ACC | 3 | 1.32 | 0.18 | 2.39 | 0.11 | -4.18 | 0.31 | |
| A59 | ACC | 3 | 1.96 | 0.22 | 4.01 | 0.11 | -5.47 | 0.31 | |

Donors

| atom name | residue. mol. # | total | x | σx | y | σy | z | σz |
|---------------|--------------------|-------|-------|------------|-------|------------|--------|------------|
| D2 | DON | 5 | -4.83 | 0.41 | 9.93 | 0.42 | -4.13 | 0.06 |
| D3 | DON | 5 | -2.29 | 0.33 | 9.76 | 0.48 | -2.96 | 0.18 |
| D6 (A8) | DON | 5 | -3.86 | 0.33 | 10.11 | 0.12 | 2.13 | 0.21 |
| D11 (A11) | DON | 5 | -3.42 | 0.36 | 10.75 | 0.31 | 6.12 | 0.36 |
| D15 (A14) | DON | 5 | -7.65 | 0.42 | 8.35 | 0.28 | 7.93 | 0.19 |
| D17 | DON | 5 | -4.88 | 0.29 | 7.13 | 0.34 | 9.26 | 0.08 |
| D21 (A42) | DON | 5 | -4.42 | 0.74 | 4.02 | 0.11 | 7.28 | 0.39 |
| D22 | DON | 5 | -5.81 | 0.30 | 1.79 | 0.28 | 0.94 | 0.10 |
| D24 | DON | 5 | -5.85 | 0.17 | -2.29 | 0.15 | -2.39 | 0.10 |
| D26 | DON | 5 | -1.59 | 0.17 | -1.52 | 0.26 | -1.17 | 0.14 |
| D27 | DON | 1 | -0.90 | - | 2.47 | - | 1.79 | - |
| D32 | DON | 5 | -5.76 | 0.30 | 3.99 | 0.12 | -5.84 | 0.34 |
| D35 (A32) | DON | 5 | -3.37 | 0.49 | 3.38 | 0.29 | -11.88 | 0.27 |
| D36 | DON | 5 | -1.89 | 0.69 | 6.00 | 0.37 | -11.25 | 0.14 |
| D43 | DON | 5 | 0.35 | 0.44 | 0.04 | 0.54 | -12.44 | 0.04 |
| D47 | DON | 4 | -7.47 | 0.24 | 1.06 | 0.13 | -9.91 | 0.26 |
| D49 (A43) | DON | 4 | -3.07 | 0.46 | 1.67 | 0.40 | 1.87 | 0.38 |
| D64 | DON | 3 | 0.37 | 0.27 | 4.92 | 0.07 | -3.02 | 0.15 |
| D65 (A55) | DON | 3 | 0.11 | 0.37 | 1.66 | 0.18 | -0.35 | 0.22 |
| Waters | | | | | | | | |
| atom name | residue. mol. # | total | x | σx | y | σy | z | σz |
| W1 | WAT | 4 | 0.62 | 0.21 | -3.17 | 0.55 | -8.81 | 0.66 |
| W9 | WAT | 4 | 2.90 | 0.30 | 3.03 | 0.33 | -8.84 | 0.37 |

Table 7C: NAD(P) Conformer Model

| atom name | total | x | σx | y | σy | z | σz |
|-----------|-------|-------|------------|-------|------------|-------|------------|
| PA | 5 | -3.59 | 0.07 | 1.15 | 0.06 | -3.16 | 0.09 |
| O1A | 5 | -3.91 | 0.07 | -0.06 | 0.08 | -2.37 | 0.06 |
| O2A | 5 | -4.70 | 0.10 | 1.87 | 0.11 | -3.82 | 0.09 |

| | | | | | | | |
|------|---|-------|------|-------|------|--------|------|
| O5'A | 5 | -2.52 | 0.10 | 0.72 | 0.06 | -4.25 | 0.09 |
| C5'A | 5 | -1.97 | 0.11 | 1.62 | 0.06 | -5.21 | 0.09 |
| C4'A | 5 | -1.00 | 0.13 | 0.82 | 0.07 | -6.06 | 0.07 |
| O4'A | 5 | -1.74 | 0.17 | -0.16 | 0.08 | -6.80 | 0.06 |
| C3'A | 5 | -0.24 | 0.20 | 1.65 | 0.08 | -7.07 | 0.11 |
| O3'A | 5 | 1.09 | 0.17 | 1.16 | 0.21 | -7.14 | 0.19 |
| C2'A | 5 | -0.96 | 0.21 | 1.42 | 0.12 | -8.38 | 0.08 |
| O2'A | 5 | -0.03 | 0.25 | 1.44 | 0.24 | -9.46 | 0.12 |
| C1'A | 5 | -1.49 | 0.16 | 0.01 | 0.09 | -8.20 | 0.07 |
| N9A | 5 | -2.74 | 0.16 | -0.23 | 0.11 | -8.94 | 0.08 |
| C8A | 5 | -3.87 | 0.15 | 0.51 | 0.05 | -9.04 | 0.13 |
| N7A | 5 | -4.77 | 0.16 | -0.07 | 0.05 | -9.80 | 0.19 |
| C5A | 5 | -4.20 | 0.14 | -1.23 | 0.09 | -10.20 | 0.13 |
| C6A | 5 | -4.67 | 0.20 | -2.26 | 0.14 | -11.02 | 0.14 |
| N6A | 5 | -5.88 | 0.24 | -2.27 | 0.19 | -11.55 | 0.20 |
| N1A | 5 | -3.84 | 0.23 | -3.30 | 0.17 | -11.24 | 0.14 |
| C2A | 5 | -2.64 | 0.22 | -3.33 | 0.19 | -10.69 | 0.18 |
| N3A | 5 | -2.13 | 0.23 | -2.39 | 0.17 | -9.90 | 0.15 |
| C4A | 5 | -2.94 | 0.14 | -1.35 | 0.12 | -9.67 | 0.08 |
| O3 | 5 | -2.67 | 0.10 | 2.02 | 0.11 | -2.19 | 0.13 |
| PN | 5 | -2.64 | 0.33 | 3.48 | 0.09 | -1.61 | 0.18 |
| O2N | 5 | -1.78 | 0.43 | 3.39 | 0.25 | -0.42 | 0.27 |
| O1N | 5 | -2.28 | 0.39 | 4.43 | 0.23 | -2.64 | 0.37 |
| O5'N | 5 | -4.08 | 0.45 | 3.75 | 0.33 | -1.10 | 0.12 |
| C5'N | 5 | -5.08 | 0.40 | 4.38 | 0.23 | -1.89 | 0.10 |
| C4'N | 5 | -5.43 | 0.23 | 5.74 | 0.13 | -1.36 | 0.03 |
| O4'N | 5 | -5.93 | 0.16 | 5.65 | 0.12 | -0.02 | 0.04 |
| C3'N | 5 | -4.26 | 0.18 | 6.68 | 0.23 | -1.23 | 0.10 |
| O3'N | 5 | -3.85 | 0.24 | 7.22 | 0.37 | -2.47 | 0.14 |
| C2'N | 5 | -4.83 | 0.19 | 7.72 | 0.11 | -0.32 | 0.12 |
| O2'N | 5 | -5.69 | 0.24 | 8.58 | 0.11 | -1.05 | 0.14 |
| C1'N | 5 | -5.61 | 0.09 | 6.86 | 0.10 | 0.66 | 0.03 |
| N1N | 5 | -4.82 | 0.08 | 6.56 | 0.06 | 1.86 | 0.06 |
| C2N | 5 | -5.21 | 0.09 | 7.16 | 0.08 | 3.04 | 0.07 |

| | | | | | | |
|-----|-------|------|------|------|--------|------|
| C3N | -4.46 | 0.11 | 6.94 | 0.05 | 4.21 | 0.09 |
| C7N | -4.88 | 0.17 | 7.54 | 0.12 | 5.51 | 0.09 |
| O7N | -4.17 | 0.19 | 7.45 | 0.25 | 6.50 | 0.12 |
| N7N | -6.04 | 0.21 | 8.19 | 0.19 | 5.56 | 0.07 |
| C4N | -3.34 | 0.13 | 6.14 | 0.07 | 4.16 | 0.09 |
| C5N | -2.95 | 0.14 | 5.55 | 0.14 | 2.98 | 0.11 |
| C6N | -3.70 | 0.10 | 5.76 | 0.14 | 1.84 | 0.10 |
| P2' | -0.06 | 0.34 | 2.60 | 0.41 | -10.53 | 0.12 |
| OP1 | -0.57 | 0.66 | 3.20 | 0.94 | -10.55 | 0.97 |
| OP2 | 0.89 | 1.15 | 2.72 | 0.92 | -10.83 | 0.65 |
| OP3 | -0.55 | 0.81 | 2.71 | 0.77 | -11.09 | 0.69 |

Table 7D: Polypeptide and Solvent Interactors

| | | | | | | | | | |
|------------|------------|-----------|----------|---------------|----------------|---------------|-----------------|---------------|----------------|
| A8 | ACC | 8 | 5 | -3.864 | 0.33454 | 10.112 | 0.123743 | 2.1286 | 0.21329 |
| NE2 | HIS 1 | 108 | | -3.007 | | 10.311 | | 6.445 | |
| NE2 | HIS 2 | 117 | | -3.912 | | 10.677 | | 6.566 | |
| NE2 | HIS 3 | 110 | | -3.39 | | 11.167 | | 5.845 | |
| NE2 | HIS 4 | 110 | | -3.153 | | 10.889 | | 5.871 | |
| NE2 | HIS 5 | 110 | | -3.636 | | 10.73 | | 5.849 | |
| A11 | ACC | 11 | 5 | -3.42 | 0.36451 | 10.755 | 0.312868 | 6.1152 | 0.35899 |
| OG | SER 1 | 139 | | -7.14 | | 8.138 | | 8.261 | |
| OG | SER 2 | 166 | | -8.27 | | 7.971 | | 7.92 | |
| OG | SER 3 | 159 | | -7.772 | | 8.621 | | 7.778 | |
| OG | SER 4 | 159 | | -7.65 | | 8.495 | | 7.82 | |
| OG | SER 5 | 159 | | -7.437 | | 8.529 | | 7.856 | |
| A14 | ACC | 14 | 5 | -7.654 | 0.41973 | 8.3508 | 0.280664 | 7.927 | 0.19384 |
| OE1 | GLN 1 | 161 | | -7.73 | | 7.828 | | 3.644 | |
| OE1 | GLN 2 | 190 | | -8.407 | | 7.736 | | 3.471 | |
| OE1 | GLN 3 | 183 | | -8.012 | | 8.025 | | 3.461 | |
| OE1 | GLN 4 | 183 | | -8.028 | | 7.965 | | 3.514 | |
| OE1 | GLN 5 | 183 | | -8.175 | | 7.938 | | 3.638 | |
| A18 | ACC | 18 | 5 | -8.07 | 0.24765 | 7.8984 | 0.1155 | 3.5456 | 0.08936 |
| OG | SER 1 | 233 | | -2.688 | | 3.039 | | -11.94 | |
| OG | SER 2 | 271 | | -3.273 | | 3.123 | | -12.31 | |
| OG | SER 3 | 263 | | -3.404 | | 3.664 | | -11.79 | |
| OG | SER 4 | 263 | | -3.447 | | 3.654 | | -11.8 | |
| OG | SER 5 | 263 | | -4.061 | | 3.397 | | -11.59 | |
| A32 | ACC | 32 | 5 | -3.375 | 0.48964 | 3.3754 | 0.290794 | -11.88 | 0.27029 |
| OE1 | GLU 1 | 241 | | -6.654 | | -3.242 | | -15.12 | |
| OE1 | GLU 2 | 279 | | -6.05 | | -4.113 | | -15.74 | |
| OE1 | GLU 3 | 271 | | -6.813 | | -3.347 | | -15.07 | |
| OE1 | GLU 4 | 271 | | -6.579 | | -3.598 | | -15.29 | |
| OE1 | GLU 5 | 271 | | -7.419 | | -3.871 | | -15.4 | |
| A37 | ACC | 37 | 5 | -6.703 | 0.49217 | -3.634 | 0.361573 | -15.32 | 0.26598 |
| OE2 | GLU 1 | 241 | | -7.599 | | -4.219 | | -13.37 | |
| OE2 | GLU 2 | 279 | | -6.79 | | -4.645 | | -13.74 | |

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230

| | | | | | | |
|------------|------------|-----------|----------|---------------|----------------|----------------|
| OE2 | GLU | 3 | 271 | -7.422 | -4.351 | -13.25 |
| OE2 | GLU | 4 | 271 | -7.243 | -4.266 | -13.32 |
| OE2 | GLU | 5 | 271 | -7.176 | -4.27 | -13.3 |
| A38 | ACC | 38 | 5 | -7.246 | 0.30349 | -4.35 |
| OD1 | ASN | 1 | 242 | -8.167 | -0.847 | -11.28 |
| OD1 | ASN | 3 | 272 | -8.198 | -0.802 | -10.63 |
| OD1 | ASN | 4 | 272 | -8.082 | -0.656 | -10.87 |
| OD1 | ASN | 5 | 272 | -8.588 | -0.828 | -10.63 |
| A40 | ACC | 40 | 4 | -8.259 | 0.22491 | -0.783 |
| OH | TYR | 2 | 216 | -4.48 | 3.904 | 7.523 |
| OH | TYR | 3 | 209 | -4.079 | 3.966 | 7.44 |
| OH | TYR | 4 | 209 | -4.093 | 4.039 | 7.418 |
| OH | TYR | 5 | 209 | -3.784 | 3.971 | 7.417 |
| A42 | ACC | 42 | 4 | -4.109 | 0.28544 | 3.97 |
| SG | CYS | 2 | 217 | -2.381 | 1.081 | 2.263 |
| OG | SER | 3 | 210 | -3.198 | 1.802 | 1.827 |
| OG | SER | 4 | 210 | -3.328 | 1.843 | 2.013 |
| OG | SER | 5 | 210 | -3.366 | 1.953 | 1.365 |
| A43 | ACC | 43 | 4 | -3.068 | 0.46378 | 1.6698 |
| OG | SER | 3 | 214 | 0.302 | 1.569 | -0.171 |
| OG | SER | 4 | 214 | 0.348 | 1.533 | -0.286 |
| OG | SER | 5 | 214 | -0.31 | 1.864 | -0.589 |
| A55 | ACC | 55 | 3 | 0.1133 | 0.36734 | 1.6553 |
| OD1 | ASP | 3 | 216 | 1.445 | 2.279 | -4.029 |
| OD1 | ASP | 4 | 216 | 1.393 | 2.409 | -3.965 |
| OD1 | ASP | 5 | 216 | 1.107 | 2.494 | -4.537 |
| A58 | ACC | 58 | 3 | 1.315 | 0.182 | 2.394 |
| OD2 | ASP | 3 | 216 | 2.06 | 3.9 | -5.346 |
| OD2 | ASP | 4 | 216 | 2.112 | 3.991 | -5.233 |
| OD2 | ASP | 5 | 216 | 1.712 | 4.127 | -5.826 |
| A59 | ACC | | | | | 0.31486 |

Donors

| atom name | residue mol. # | residue # | total x | σx | y | σy | z | σz |
|-----------|----------------|-----------|---------|----------------|-----------------|----------------|--------|----|
| N | VAL 1 | 21 | -4.573 | | 10.277 | | -4.214 | |
| N | THR 2 | 23 | -4.955 | | 10.482 | | -4.051 | |
| N | THR 3 | 19 | -4.601 | | 9.587 | | -4.125 | |
| N | THR 4 | 19 | -4.539 | | 9.637 | | -4.107 | |
| N | THR 5 | 19 | -5.495 | | 9.654 | | -4.137 | |
| D2 | DON | 2 | 5 | -4.833 0.40651 | 9.9274 0.419748 | -4.127 0.05884 | | |
| N | PHE 1 | 22 | -2.163 | | 9.689 | | -2.98 | |
| N | THR 2 | 24 | -2.234 | | 10.595 | | -3.208 | |
| N | TRP 3 | 20 | -2.126 | | 9.537 | | -2.765 | |
| N | TRP 4 | 20 | -2.061 | | 9.403 | | -2.815 | |
| N | TRP 5 | 20 | -2.861 | | 9.571 | | -3.033 | |
| D3 | DON | 3 | 5 | -2.289 0.32582 | 9.759 0.47832 | -2.96 0.17768 | | |
| OH | TYR 1 | 50 | -3.489 | | 9.992 | | 2.109 | |
| OH | TYR 2 | 55 | -4.193 | | 10.25 | | 2.441 | |
| OH | TYR 3 | 48 | -3.749 | | 9.978 | | 2.218 | |
| OH | TYR 4 | 48 | -3.652 | | 10.133 | | 1.976 | |
| OH | TYR 5 | 48 | -4.239 | | 10.209 | | 1.899 | |
| D6 | DON | 6 | 5 | -3.864 0.33454 | 10.112 0.123743 | 2.1286 0.21329 | | |
| HIS 1 | HIS 1 | 108 | -3.007 | | 10.311 | | 6.445 | |
| NE2 | HIS 2 | 117 | -3.912 | | 10.677 | | 6.566 | |
| NE2 | HIS 3 | 110 | -3.39 | | 11.167 | | 5.845 | |
| NE2 | HIS 4 | 110 | -3.153 | | 10.889 | | 5.871 | |
| NE2 | HIS 5 | 110 | -3.636 | | 10.73 | | 5.849 | |
| D11 | DON | 11 | 5 | -3.42 0.36451 | 10.755 0.312868 | 6.1152 0.35899 | | |
| OG | SER 1 | 139 | -7.14 | | 8.138 | | 8.261 | |
| OG | SER 2 | 166 | -8.27 | | 7.971 | | 7.92 | |
| OG | SER 3 | 159 | -7.772 | | 8.621 | | 7.778 | |
| OG | SER 4 | 159 | -7.65 | | 8.495 | | 7.82 | |
| OG | SER 5 | 159 | -7.437 | | 8.529 | | 7.856 | |
| D15 | DON | 15 | 5 | -7.654 0.41973 | 8.3508 0.280664 | 7.927 0.19384 | | |
| ND2 | ASN 1 | 140 | -4.533 | | 6.58 | | 9.266 | |

| | | | | |
|-----|------------|-----|-----------------|---------------------------------------------------------------------------|
| ND2 | ASN 2 | 167 | -5.286 | 7.047 |
| ND2 | ASN 3 | 160 | -4.994 | 7.442 |
| ND2 | ASN 4 | 160 | -4.894 | 7.259 |
| ND2 | ASN 5 | 160 | -4.669 | 7.311 |
| D17 | DON | 17 | 5 -4.875 | 0.29276 7.1278 0.33768 |
| NE1 | TRP 1 | 187 | -5.659 | 4.197 |
| OH | TYR 2 | 216 | -4.48 | 3.904 |
| OH | TYR 3 | 209 | -4.079 | 3.966 |
| OH | TYR 4 | 209 | -4.093 | 4.039 |
| OH | TYR 5 | 209 | -3.784 | 3.971 |
| D21 | DON | 21 | 5 -4.419 | 0.73594 4.0154 0.112202 7.2782 0.38549 |
| N | GLY 1 | 188 | -5.543 | 1.806 |
| N | CYS 2 | 217 | -5.457 | 1.307 |
| N | SER 3 | 210 | -5.913 | 2.008 |
| N | SER 4 | 210 | -5.995 | 1.926 |
| N | SER 5 | 210 | -6.138 | 1.889 |
| D22 | DON | 22 | 5 -5.809 | 0.29509 1.7872 0.278086 0.9352 0.09986 |
| N | LEU 1 | 190 | -6.122 | -2.167 |
| N | LEU 2 | 219 | -5.697 | -2.431 |
| N | LEU 3 | 212 | -5.848 | -2.116 |
| N | LEU 4 | 212 | -5.837 | -2.313 |
| N | LEU 5 | 212 | -5.738 | -2.444 |
| D24 | DON | 24 | 5 -5.848 | 0.1659 -2.294 0.149535 -2.392 0.10273 |
| N | GLN 1 | 192 | -1.835 | -1.942 |
| N | SER 2 | 221 | -1.633 | -1.501 |
| N | SER 3 | 214 | -1.557 | -1.387 |
| N | SER 4 | 214 | -1.543 | -1.524 |
| N | SER 5 | 214 | -1.368 | -1.233 |
| D26 | DON | 26 | 5 -1.587 | 0.16913 -1.517 0.263858 -1.173 0.1412 |
| NE2 | GLN 1 | 192 | -0.903 | 2.473 |
| D27 | DON | 27 | 1 -0.903 | 2.473 |
| N | LYS 1 | 232 | -5.402 | 4.166 |
| N | ARG 2 | 270 | -5.952 | 3.855 |

| | | | | | |
|-----|-------|-----|-----------------|----------------|------------------------|
| N | LYS 3 | 262 | -5.685 | 4.007 | -5.639 |
| N | LYS 4 | 262 | -5.623 | 3.992 | -5.582 |
| N | LYS 5 | 262 | -6.162 | 3.913 | -5.584 |
| D32 | DON | 32 | 5 -5.765 | 0.29619 | 3.9866 0.117649 |
| OG | SER 1 | 233 | -2.688 | 3.039 | -11.94 |
| OG | SER 2 | 271 | -3.273 | 3.123 | -12.31 |
| OG | SER 3 | 263 | -3.404 | 3.664 | -11.79 |
| OG | SER 4 | 263 | -3.447 | 3.654 | -11.8 |
| OG | SER 5 | 263 | -4.061 | 3.397 | -11.59 |
| D35 | DON | 35 | 5 -3.375 | 0.48964 | 3.3754 0.290794 |
| N | VAL 1 | 234 | -1.14 | 5.556 | -11.43 |
| N | PHE 2 | 272 | -1.614 | 5.656 | -11.37 |
| N | VAL 3 | 264 | -1.81 | 6.206 | -11.19 |
| N | VAL 4 | 264 | -1.882 | 6.219 | -11.12 |
| N | VAL 5 | 264 | -3.012 | 6.373 | -11.15 |
| D36 | DON | 36 | 5 -1.892 | 0.68993 | 6.002 0.369113 |
| NH1 | ARG 1 | 238 | 0.069 | -0.686 | -12 |
| NH2 | ARG 2 | 276 | 1.098 | 0.722 | -13.92 |
| NH1 | ARG 3 | 268 | 0.415 | 0.209 | -12.73 |
| NH1 | ARG 4 | 268 | 0.039 | -0.27 | -11.5 |
| NH2 | ARG 5 | 268 | 0.142 | 0.24 | -12.05 |
| D43 | DON | 43 | 4 0.3526 | 0.44234 | 0.043 0.537777 |
| ND2 | ASN 1 | 242 | -7.301 | 0.978 | -10.22 |
| ND2 | ASN 3 | 272 | -7.385 | 1.094 | -9.791 |
| ND2 | ASN 4 | 272 | -7.367 | 1.218 | -10.01 |
| ND2 | ASN 5 | 272 | -7.832 | 0.939 | -9.618 |
| D47 | DON | 47 | 4 -7.471 | 0.2432 | 1.0573 0.125771 |
| SG | CYS 2 | 217 | -2.381 | 1.081 | 2.263 |
| OG | SER 3 | 210 | -3.198 | 1.802 | 1.827 |
| OG | SER 4 | 210 | -3.328 | 1.843 | 2.013 |
| OG | SER 5 | 210 | -3.366 | 1.953 | 1.365 |
| D49 | DON | 49 | 4 -3.068 | 0.46378 | 1.6698 0.397644 |
| NZ | LYS 3 | 21 | 0.563 | 4.894 | -2.898 |

| | | | | | | |
|------------|------------|-----------|---|-------------|----------------|---------------|
| NZ | LYS 4 | 21 | | 0.487 | 4.857 | -2.975 |
| NZ | LYS 5 | 21 | | 0.06 | 4.999 | -3.187 |
| D64 | DON | 64 | 3 | 0.37 | 0.27114 | 4.9167 |
| OG | SER 3 | 214 | | 0.302 | 1.569 | -0.171 |
| OG | SER 4 | 214 | | 0.348 | 1.533 | -0.286 |
| OG | SER 5 | 214 | | -0.31 | 1.864 | -0.589 |
| D65 | DON | 65 | 3 | 0.1133 | 0.36734 | 1.6553 |
| | | | | 0.181605 | -0.349 | 0.21593 |

| atom name | residue mol. # | residue # | total | x | σx | y | σy | z | σz |
|-----------|----------------|-----------|-------|--------------|----------------|--------------|-----------------|---------------|----------------|
| O | HOH 1 | 396 | | 3.263 | 2.796 | | | -9.047 | |
| O | HOH 3 | 536 | | 3.02 | 2.698 | | | -8.645 | |
| O | HOH 4 | 484 | | 2.686 | 3.261 | | | -8.435 | |
| O | HOH 5 | 586 | | 2.613 | 3.35 | | | -9.237 | |
| W9 | WAT | 9 | 4 | 2.895 | 0.30235 | 3.026 | 0.326948 | -8.841 | 0.36629 |
| O | HOH 1 | 307 | | 0.306 | -3.84 | | | -7.869 | |
| O | HOH 3 | 731 | | 0.694 | -3.294 | | | -8.887 | |
| O | HOH 4 | 485 | | 0.782 | -3.008 | | | -9.378 | |
| O | HOH 5 | 483 | | 0.686 | -2.519 | | | -9.123 | |
| W1 | WAT | 1 | 4 | 0.617 | 0.21185 | -3.165 | 0.552036 | -8.814 | 0.66129 |

Table 8A: Pharmacophore family 6 Subset

| Molecule # | pdb | type | RMSD from Family Avg. |
|------------|------|-----------------------------------------------------|--------------------------------|
| 1 | 1AI9 | Dihydrofolate Reductase (<i>candida albicans</i>) | 0.49 |
| 2 | 1DAJ | DHFR (pneumocystis carinii) | 0.8 |
| 3 | 1DLR | DHFR (human) | 0.6 |
| 4 | 1DR1 | DHFR (chicken) | 0.83 |
| 5 | 1DRE | DHFR (<i>E. coli</i>) | 0.91 |
| 6 | 3DFR | DHFR (<i>Lactobacillus casei</i>) | 0.84 |

Table 8B: Polypeptide and solvent Interactors (average coordinates)

| Acceptors | | Name | total | x | y | σ_x | σ_y | z | σ_z |
|-----------|------|------|-------|-------|------|------------|------------|-------|------------|
| atom name | name | | | | | | | | |
| A2 | ACC | | 6 | -7.76 | 0.34 | 9.50 | 0.60 | 15.24 | 0.31 |
| A3 | ACC | | 6 | -3.33 | 0.36 | 9.00 | 0.28 | 13.41 | 0.29 |
| A7 | ACC | | 6 | 4.38 | 0.42 | 8.51 | 0.59 | 14.79 | 0.44 |
| A8 | ACC | | 5 | 0.64 | 0.44 | 10.67 | 0.55 | 12.99 | 0.29 |
| A22 | ACC | | 5 | 1.78 | 0.52 | -12.11 | 0.61 | 17.27 | 0.35 |
| A29 | ACC | | 3 | 1.38 | 0.22 | -3.65 | 0.98 | 10.30 | 0.42 |
| A45 (D53) | ACC | | 5 | 7.52 | 0.32 | -6.82 | 0.15 | 17.60 | 0.52 |
| A64 | ACC | | 1 | 3.88 | | 7.64 | | 10.73 | |
| Donors | | Name | total | x | y | σ_x | σ_y | z | σ_z |
| atom name | name | | | | | | | | |
| D2 | DON | | 6 | -8.77 | 0.24 | 8.47 | 0.48 | 17.58 | 0.39 |
| D5 | DON | | 6 | 0.31 | 0.46 | 10.32 | 0.28 | 10.41 | 0.31 |
| D7 | DON | | 6 | 4.49 | 0.64 | 8.48 | 0.37 | 11.28 | 0.47 |
| D8 | DON | | 6 | 3.29 | 0.49 | 9.75 | 0.37 | 13.31 | 0.28 |
| D10 | DON | | 6 | 0.75 | 0.68 | 11.75 | 0.20 | 14.90 | 0.31 |

| | | | | | | | | |
|-----------|-----|---|-------|------|-------|------|-------|------|
| D13 | DON | 6 | 0.42 | 0.31 | -1.68 | 0.29 | 18.99 | 0.21 |
| D14 | DON | 6 | 3.77 | 0.31 | -2.26 | 0.30 | 17.84 | 0.28 |
| D15 | DON | 3 | 9.09 | 0.30 | -3.80 | 0.34 | 14.68 | 0.76 |
| D18 | DON | 6 | 4.89 | 0.37 | 0.01 | 0.38 | 16.50 | 0.32 |
| D19 | DON | 3 | 5.76 | 0.34 | -0.45 | 1.23 | 11.73 | 0.54 |
| D20 | DON | 6 | 3.21 | 0.48 | 2.15 | 0.27 | 17.41 | 0.31 |
| D24 | DON | 6 | 8.21 | 0.50 | -9.32 | 0.64 | 16.12 | 0.77 |
| D25 | DON | 6 | 5.73 | 0.39 | -9.28 | 0.30 | 16.15 | 0.47 |
| D27 | DON | 2 | 4.63 | 0.21 | -8.88 | 0.26 | 11.81 | 0.22 |
| D35 | DON | 6 | -1.87 | 0.34 | 0.75 | 0.49 | 16.42 | 0.33 |
| D37 | DON | 6 | -2.91 | 0.56 | -1.48 | 0.83 | 11.81 | 0.33 |
| D38 | DON | 6 | -3.30 | 0.47 | -3.07 | 0.64 | 14.06 | 0.39 |
| D40 | DON | 5 | -6.32 | 0.26 | 3.86 | 0.48 | 17.78 | 0.67 |
| D53 (A45) | DON | 5 | 7.52 | 0.32 | -6.82 | 0.15 | 17.60 | 0.52 |
| D58 | DON | 2 | 4.59 | 0.01 | 4.70 | 0.53 | 10.76 | 0.38 |

Waters

| atom name | Name | total | x | σx | y | σy | z | σz |
|-----------|------|-------|-------|------|--------|------|-------|------|
| W5 | WAT | 3 | 3.12 | 0.69 | 4.35 | 0.33 | 10.23 | 0.39 |
| W7 | WAT | 3 | 2.33 | 0.11 | 6.97 | 0.14 | 10.21 | 0.07 |
| W9 | WAT | 2 | 1.38 | 0.94 | 3.27 | 0.01 | 9.07 | 0.57 |
| W10 | WAT | 3 | -2.58 | 0.27 | -11.63 | 0.89 | 15.29 | 0.33 |

Table 8C: NAD(P) Conformer Model

| atom name | total | x | σx | y | σy | z | σz |
|-----------|-------|-------|------|-------|------|-------|------|
| PA | 6 | 1.05 | 0.24 | -0.17 | 0.19 | 14.67 | 0.19 |
| O1A | 6 | 1.19 | 0.24 | 0.64 | 0.25 | 15.88 | 0.23 |
| O2A | 6 | -0.20 | 0.24 | -0.90 | 0.28 | 14.47 | 0.18 |
| O5'A | 6 | 2.35 | 0.21 | -1.13 | 0.14 | 14.56 | 0.24 |
| C5'A | 6 | 2.40 | 0.23 | -2.23 | 0.10 | 13.62 | 0.23 |
| C4'A | 6 | 3.42 | 0.23 | -3.27 | 0.14 | 14.17 | 0.18 |
| O4'A | 6 | 2.79 | 0.36 | -3.93 | 0.29 | 15.07 | 0.24 |

| | | | | | | | |
|------|---|-------|------|-------|------|-------|------|
| C3'A | 6 | 3.64 | 0.12 | -4.36 | 0.13 | 13.07 | 0.19 |
| O3'A | 6 | 4.70 | 0.13 | -3.76 | 0.25 | 12.26 | 0.24 |
| C2'A | 6 | 4.06 | 0.05 | -5.51 | 0.17 | 14.00 | 0.26 |
| O2'A | 6 | 5.31 | 0.06 | -5.32 | 0.34 | 14.57 | 0.28 |
| C1'A | 6 | 3.05 | 0.11 | -5.32 | 0.22 | 15.11 | 0.22 |
| N9A | 6 | 1.81 | 0.09 | -5.96 | 0.35 | 14.84 | 0.21 |
| C8A | 6 | 0.76 | 0.17 | -5.40 | 0.56 | 14.27 | 0.47 |
| N7A | 6 | -0.27 | 0.17 | -6.16 | 0.65 | 14.17 | 0.44 |
| C5A | 6 | 0.21 | 0.15 | -7.35 | 0.53 | 14.68 | 0.21 |
| C6A | 6 | -0.44 | 0.24 | -8.68 | 0.51 | 14.89 | 0.32 |
| N6A | 6 | -1.69 | 0.28 | -8.92 | 0.67 | 14.53 | 0.44 |
| N1A | 6 | 0.29 | 0.35 | -9.56 | 0.36 | 15.44 | 0.49 |
| C2A | 6 | 1.54 | 0.34 | -9.19 | 0.25 | 15.79 | 0.52 |
| N3A | 6 | 2.22 | 0.25 | -8.09 | 0.22 | 15.65 | 0.34 |
| C4A | 6 | 1.45 | 0.13 | -7.18 | 0.35 | 15.09 | 0.07 |
| O3 | 6 | 1.42 | 0.24 | 0.75 | 0.10 | 13.47 | 0.20 |
| PN | 6 | 0.72 | 0.34 | 1.45 | 0.19 | 12.25 | 0.14 |
| O1N | 6 | 1.73 | 0.45 | 1.89 | 0.29 | 11.31 | 0.22 |
| O2N | 6 | -0.36 | 0.53 | 0.71 | 0.34 | 11.74 | 0.15 |
| O5'N | 6 | 0.22 | 0.15 | 2.75 | 0.17 | 12.92 | 0.26 |
| C5'N | 6 | 1.01 | 0.12 | 3.77 | 0.28 | 13.48 | 0.39 |
| C4'N | 6 | 0.38 | 0.25 | 5.08 | 0.27 | 13.02 | 0.22 |
| O4'N | 6 | -0.91 | 0.16 | 5.18 | 0.29 | 13.67 | 0.13 |
| C3'N | 6 | 1.12 | 0.29 | 6.33 | 0.23 | 13.52 | 0.32 |
| O3'N | 6 | 1.00 | 0.36 | 7.39 | 0.27 | 12.63 | 0.36 |
| C2'N | 6 | 0.45 | 0.21 | 6.61 | 0.24 | 14.87 | 0.28 |
| O2'N | 6 | 0.66 | 0.31 | 7.95 | 0.27 | 15.21 | 0.40 |
| C1'N | 6 | -0.96 | 0.21 | 6.30 | 0.20 | 14.54 | 0.23 |
| N1N | 6 | -1.94 | 0.08 | 6.13 | 0.21 | 15.69 | 0.16 |
| C2N | 6 | -3.04 | 0.10 | 6.97 | 0.25 | 15.83 | 0.15 |
| C3N | 6 | -3.94 | 0.11 | 6.79 | 0.28 | 16.76 | 0.16 |
| C7N | 6 | -5.03 | 0.17 | 7.76 | 0.42 | 16.79 | 0.23 |
| O7N | 6 | -5.87 | 0.22 | 7.55 | 0.50 | 17.62 | 0.42 |
| N7N | 6 | -5.15 | 0.38 | 8.68 | 0.43 | 15.88 | 0.20 |

Table 8D: Polypeptide and Solvent Interactors

| Acceptors | atom name | residue mol. # | residue # | total | x | σx | y | σy | z | σz |
|-----------|-----------|----------------|-----------|-------|------|------------|------|------------|-------|------------|
| C4N | O | ALA 1 | 11 | -8.25 | | 9.15 | | | 15.70 | |
| C5N | O | ALA 2 | 12 | -7.62 | | 9.56 | | | 15.25 | |
| C6N | O | ALA 3 | 9 | -7.84 | | 8.91 | | | 15.02 | |
| P2' | O | ALA 4 | 9 | -8.02 | | 9.04 | | | 15.08 | |
| OP1 | O | ALA 5 | 7 | -7.34 | | 10.51 | | | 14.88 | |
| OP2 | O | ALA 6 | 6 | -7.50 | | 9.83 | | | 15.51 | |
| OP3 | A2 | ACC 2 | 6 | -7.76 | 0.34 | 9.50 | 0.60 | | 15.24 | 0.31 |
| | O | ILE 1 | 19 | -3.73 | | 9.16 | | | 13.34 | |
| | O | ILE 2 | 19 | -3.77 | | 8.82 | | | 13.73 | |
| | O | ILE 3 | 16 | -3.18 | | 8.72 | | | 13.35 | |
| | O | ILE 4 | 16 | -3.34 | | 8.72 | | | 13.44 | |
| | O | ILE 5 | 14 | -2.92 | | 9.18 | | | 12.93 | |
| | O | ILE 6 | 13 | -3.03 | | 9.39 | | | 13.70 | |
| | A3 | ACC 3 | 6 | -3.33 | 0.36 | 9.00 | 0.28 | | 13.41 | 0.29 |
| | O | GLY 1 | 23 | 3.59 | | 8.74 | | | 14.29 | |
| | O | ASN 2 | 23 | 4.73 | | 8.14 | | | 14.25 | |
| | O | GLY 3 | 20 | 4.28 | | 9.37 | | | 15.16 | |
| | O | GLY 4 | 20 | 4.43 | | 8.68 | | | 14.84 | |
| | O | ASN 5 | 18 | 4.63 | | 8.52 | | | 15.30 | |
| | O | GLY 6 | 17 | 4.64 | | 7.62 | | | 14.92 | |

| | | | | |
|-----|------------|-----------|--------------|-------------|
| N | ALA 4 | 9 | -8.84 | 8.16 |
| N | ALA 5 | 7 | -8.61 | 9.19 |
| N | ALA 6 | 6 | -8.39 | 8.86 |
| D2 | DON | 2 | -8.77 | 0.24 |
| N | TYR 1 | 21 | -0.42 | 10.64 |
| N | ARG 2 | 21 | 0.01 | 10.40 |
| N | LYS 3 | 18 | 0.40 | 10.07 |
| N | LYS 4 | 18 | 0.32 | 9.96 |
| N | MET 5 | 16 | 0.86 | 10.62 |
| N | LYS 6 | 15 | 0.70 | 10.26 |
| D5 | DON | 5 | 0.31 | 0.46 |
| N | GLY 1 | 23 | 3.65 | 9.06 |
| N | ASN 2 | 23 | 4.05 | 8.21 |
| N | GLY 3 | 20 | 4.51 | 8.63 |
| N | GLY 4 | 20 | 4.53 | 8.63 |
| N | ASN 5 | 18 | 5.57 | 8.31 |
| N | GLY 6 | 17 | 4.61 | 8.02 |
| D7 | DON | 7 | 6 | 4.49 |
| N | LYS 1 | 24 | 2.49 | 10.14 |
| N | SER 2 | 24 | 3.18 | 9.36 |
| N | ASP 3 | 21 | 3.13 | 10.15 |
| N | ASN 4 | 21 | 3.34 | 9.95 |
| N | ALA 5 | 19 | 3.82 | 9.57 |
| N | HIS 6 | 18 | 3.78 | 9.34 |
| D8 | DON | 8 | 6 | 3.29 |
| N | MET 1 | 25 | -0.11 | 11.91 |
| N | LEU 2 | 25 | 1.21 | 11.60 |
| N | PHE 3 | 22 | 0.10 | 11.65 |
| N | LEU 4 | 22 | 0.47 | 11.75 |
| N | MET 5 | 20 | 1.42 | 12.04 |
| N | LEU 6 | 19 | 1.41 | 11.53 |
| D10 | DON | 10 | 6 | 0.75 |

| | | | | |
|-----|----|---|-------|-------|
| GLY | 1 | | 0.99 | 19.18 |
| N | 55 | | -2.06 | 19.18 |
| N | 58 | | -1.46 | 19.18 |
| N | 53 | | -1.88 | 18.67 |
| N | 53 | | 0.43 | 18.67 |
| GLY | 4 | | 0.52 | 18.78 |
| GLY | 5 | | 0.23 | 19.06 |
| GLY | 6 | | 0.14 | 19.06 |
| DON | 13 | 6 | 0.42 | 0.21 |
| N | 56 | | 0.31 | 18.99 |
| N | 59 | | 4.28 | 18.05 |
| ARG | 2 | | -2.84 | 18.05 |
| LYS | 3 | | -2.00 | 18.08 |
| LYS | 4 | | 3.60 | 17.59 |
| LYS | 5 | | 3.84 | 17.43 |
| LYS | 6 | | 3.92 | 17.84 |
| ARG | 4 | | 3.45 | 17.84 |
| ARG | 5 | | 3.51 | 18.07 |
| ARG | 6 | | -2.27 | 18.07 |
| DON | 14 | 6 | 3.77 | 0.28 |
| NH | 56 | | 0.31 | 17.84 |
| LYS | 54 | | -2.24 | 0.28 |
| LYS | 54 | | 8.78 | 15.50 |
| DON | 15 | | -3.97 | 14.54 |
| NZ | 54 | | 9.39 | 14.01 |
| NZ | 54 | | -3.41 | 14.01 |
| DON | 15 | 3 | 9.10 | 14.01 |
| NH | 56 | | -4.01 | 14.01 |
| LYS | 54 | | -3.80 | 14.68 |
| DON | 15 | | -3.97 | 16.65 |
| NZ | 57 | | 5.58 | 16.94 |
| LYS | 60 | | 4.68 | 16.22 |
| LYS | 55 | | 4.80 | 16.06 |
| LYS | 55 | | 4.95 | 16.53 |
| HIS | 45 | | 4.53 | 16.60 |
| ARG | 44 | | 4.80 | 16.60 |
| DON | 18 | 6 | 4.89 | 0.32 |
| NZ | 57 | | 0.37 | 0.32 |
| LYS | 1 | | 0.01 | 0.38 |
| HIS | 45 | | -1.79 | 11.41 |
| NE2 | | | -0.20 | 12.35 |
| NE | 44 | | 5.83 | 11.42 |
| ARG | 6 | | 5.42 | 11.42 |
| DON | 19 | 3 | 0.63 | 11.73 |
| THR | 1 | | 0.42 | 0.54 |
| N | 58 | | 1.68 | 17.55 |
| THR | 2 | | 3.07 | 17.92 |
| N | 61 | | 2.49 | 2.04 |
| THR | 3 | | 2.93 | 2.04 |
| N | 56 | | 1.11 | 17.18 |

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| | | | | | | | | | | | |
|-----|-----|----|------|-------|------|--------|------|-------|------|--|--|
| | | | | | | | | | | | |
| W5 | WAT | 5 | 3 | 3.12 | 0.69 | 4.35 | 0.33 | 10.23 | 0.39 | | |
| O | HOH | 3 | 1.94 | 2.39 | | 6.87 | | 10.29 | | | |
| O | HOH | 4 | 2.20 | 2.39 | | 7.13 | | 10.16 | | | |
| O | HOH | 6 | 2.08 | 2.21 | | 6.90 | | 10.19 | | | |
| W7 | WAT | 7 | 3 | 2.33 | 0.11 | 6.97 | 0.14 | 10.21 | 0.07 | | |
| O | HOH | 3 | 2.38 | 2.04 | | 3.26 | | 9.48 | | | |
| O | HOH | 6 | 3.01 | 0.72 | | 3.27 | | 8.67 | | | |
| W9 | WAT | 9 | 2 | 1.38 | 0.94 | 3.27 | 0.01 | 9.07 | 0.57 | | |
| O | HOH | 3 | 2.55 | -2.28 | | -11.29 | | 15.13 | | | |
| O | HOH | 4 | 4.93 | -2.82 | | -10.95 | | 15.67 | | | |
| O | HOH | 6 | 2.66 | -2.62 | | -12.63 | | 15.07 | | | |
| W10 | WAT | 10 | 3 | -2.58 | 0.27 | -11.63 | 0.89 | 15.29 | 0.33 | | |

Table 9A: Pharmacophany 7 Subset

| Molecule # | pdb | type | rmsd from Family Avg. |
|------------|------|-------------------------------------------------|--------------------------------|
| 1 | 1GET | Glutathione Reductase (E. coli) | 0.34 |
| 2 | 1GRB | Glutathione Reductase (human) | 0.66 |
| 3 | 2NPX | NADH Peroxidase (strep faecalis) | 0.82 |
| 4 | 1TDF | Thioredoxin Reductase (E. Coli) | 0.89 |
| 5 | 1TRY | Trypanothione Reductase(Critchidia fasciculata) | 2.17* |

*NAD(P) is in an inactive conformation

Table 9B: Polypeptide and Solvent Interactors (average coordinates)

| Acceptors | | residue. mol. # | total | x | cx | y | cy | z | cz |
|-----------|-----|--------------------|-------|------|-------|------|-------|------|----|
| A11 | ACC | 4 | -3.74 | 0.43 | 4.39 | 1.20 | 14.96 | 0.59 | |
| A12 | ACC | 2 | -4.46 | 0.14 | 6.91 | 0.01 | 13.10 | 0.51 | |
| A21 | ACC | 3 | -7.67 | 0.40 | -0.28 | 0.63 | 6.97 | 0.49 | |
| A27 | ACC | 5 | -6.51 | 0.79 | 8.70 | 0.33 | 10.16 | 0.42 | |
| A37 | ACC | 1 | 9.32 | - | 1.02 | - | 6.96 | - | |
| A38 | ACC | 1 | 8.04 | - | 2.39 | - | 7.96 | - | |
| A43 (D46) | ACC | 1 | -1.72 | - | 2.70 | - | 6.02 | - | |
| Donors | | residue. mol. # | total | x | cx | y | cy | z | cz |
| D8 | DON | 5 | 0.53 | 0.17 | 4.12 | 0.23 | 9.87 | 0.65 | |
| D10 | DON | 4 | -0.29 | 0.12 | 2.72 | 0.33 | 12.17 | 0.28 | |
| D13 | DON | 4 | 11.13 | 0.14 | -1.28 | 0.24 | 5.56 | 0.39 | |
| D14 | DON | 4 | 10.96 | 0.24 | -3.44 | 0.24 | 4.80 | 0.45 | |
| D15 | DON | 4 | 9.51 | 0.04 | -1.85 | 0.43 | 4.07 | 0.31 | |

| | | | | | | | | |
|-----------|-----|---|-------|------|-------|------|------|------|
| D18 | DON | 3 | 8.97 | 1.77 | 3.01 | 1.32 | 1.85 | 0.48 |
| D23 | DON | 5 | 2.38 | 0.54 | -3.84 | 0.13 | 9.65 | 0.30 |
| D46 (A43) | DON | 1 | -1.72 | - | 2.70 | - | 6.02 | - |
| D58 | DON | 1 | 3.70 | - | 2.30 | - | 3.85 | - |
| D62 | DON | 1 | -5.70 | - | 2.24 | - | 2.88 | - |

| Waters | atom name | residue mol. # | total | x | σx | y | σy | z | σz |
|--------|-----------|----------------|-------|------|-------|------|-------|------|----|
| W2 | WAT | 3 | 0.36 | 0.44 | -3.68 | 0.38 | 12.46 | 0.18 | |
| W4 | WAT | 4 | 2.93 | 0.16 | 1.13 | 0.26 | 10.91 | 0.18 | |
| W6 | WAT | 5 | -9.38 | 0.47 | 6.86 | 0.35 | 8.83 | 0.85 | |
| W10 | WAT | 2 | 0.45 | 0.22 | 3.40 | 0.19 | 5.75 | 0.60 | |
| W13 | WAT | 3 | -6.28 | 0.08 | -3.16 | 0.26 | 9.68 | 0.49 | |

Table 9C: NAD (P) Conformer Model

| atom name | total | x | σx | y | σy | z | σz |
|-----------|-------|------|------|-------|------|------|------|
| PA | 5 | 0.93 | 0.13 | -0.09 | 0.32 | 6.93 | 0.27 |
| O1A | 5 | 0.14 | 0.09 | 1.08 | 0.42 | 6.77 | 0.65 |
| O2A | 5 | 1.08 | 0.29 | -1.04 | 0.52 | 5.87 | 0.08 |
| O5'A | 5 | 2.38 | 0.11 | 0.41 | 0.17 | 7.37 | 0.16 |
| C5'A | 5 | 3.43 | 0.24 | -0.49 | 0.18 | 7.71 | 0.15 |
| C4'A | 5 | 4.73 | 0.18 | 0.09 | 0.26 | 7.34 | 0.36 |
| O4'A | 5 | 5.80 | 0.27 | -0.54 | 0.45 | 7.99 | 0.17 |
| C3'A | 5 | 5.07 | 0.14 | -0.04 | 0.62 | 5.96 | 0.38 |
| O3'A | 5 | 4.90 | 0.67 | 0.84 | 0.92 | 5.36 | 0.96 |
| C2'A | 5 | 6.35 | 0.42 | -0.33 | 0.34 | 5.72 | 0.24 |
| O2'A | 5 | 6.88 | 0.18 | 0.71 | 0.74 | 5.16 | 0.35 |
| C1'A | 5 | 6.90 | 0.27 | -0.63 | 0.31 | 7.08 | 0.22 |
| N9A | 5 | 7.56 | 0.16 | -1.93 | 0.24 | 7.16 | 0.17 |
| C8A | 5 | 7.19 | 0.18 | -3.11 | 0.27 | 6.55 | 0.20 |
| N7A | 5 | 7.98 | 0.18 | -4.12 | 0.22 | 6.87 | 0.22 |
| C5A | 5 | 8.90 | 0.17 | -3.57 | 0.15 | 7.72 | 0.19 |

"T₁ T₂ T₃ T₄ T₅ T₆ T₇ T₈ T₉ T₁₀ T₁₁ T₁₂ T₁₃ T₁₄ T₁₅ T₁₆ T₁₇ T₁₈ T₁₉ T₂₀

| | | | | | | | |
|------|---|-------|------|-------|------|-------|------|
| C6A | 5 | 10.00 | 0.19 | -4.16 | 0.07 | 8.39 | 0.21 |
| N6A | 5 | 10.34 | 0.27 | -5.42 | 0.05 | 8.23 | 0.27 |
| N1A | 5 | 10.72 | 0.16 | -3.34 | 0.07 | 9.17 | 0.23 |
| C2A | 5 | 10.42 | 0.10 | -2.04 | 0.11 | 9.27 | 0.21 |
| N3A | 5 | 9.45 | 0.10 | -1.39 | 0.13 | 8.66 | 0.19 |
| C4A | 5 | 8.68 | 0.13 | -2.21 | 0.16 | 7.90 | 0.17 |
| O3 | 5 | 0.38 | 0.10 | -0.91 | 0.20 | 8.17 | 0.20 |
| PN | 5 | -0.15 | 0.14 | -0.48 | 0.48 | 9.57 | 0.41 |
| O2N | 5 | 0.14 | 0.49 | 0.83 | 0.44 | 9.75 | 0.95 |
| O1N | 5 | 0.30 | 0.16 | -1.45 | 1.05 | 10.42 | 0.24 |
| O5'N | 5 | -1.69 | 0.09 | -0.59 | 0.27 | 9.56 | 0.17 |
| C5'N | 5 | -2.47 | 0.06 | -1.57 | 0.23 | 8.85 | 0.37 |
| C4'N | 5 | -3.70 | 0.14 | -0.94 | 0.26 | 8.22 | 0.15 |
| O4'N | 5 | -4.71 | 0.05 | -0.62 | 0.08 | 9.19 | 0.03 |
| C3'N | 5 | -3.46 | 0.22 | 0.35 | 0.46 | 7.53 | 0.17 |
| O3_N | 5 | -3.17 | 0.71 | 0.29 | 0.62 | 6.28 | 0.17 |
| C2'N | 5 | -4.65 | 0.52 | 1.11 | 0.18 | 7.65 | 0.18 |
| O2_N | 5 | -5.28 | 0.75 | 0.98 | 0.55 | 6.52 | 0.28 |
| C1'N | 5 | -5.38 | 0.18 | 0.60 | 0.07 | 8.82 | 0.16 |
| N1N | 5 | -5.34 | 0.08 | 1.60 | 0.06 | 9.91 | 0.18 |
| C2N | 5 | -5.97 | 0.21 | 2.80 | 0.05 | 9.75 | 0.25 |
| C3N | 5 | -5.93 | 0.17 | 3.83 | 0.08 | 10.68 | 0.26 |
| C7N | 5 | -6.64 | 0.26 | 5.15 | 0.08 | 10.42 | 0.36 |
| O7N | 5 | -7.25 | 0.57 | 5.32 | 0.37 | 9.88 | 1.12 |
| N7N | 5 | -6.58 | 0.34 | 6.07 | 0.28 | 10.81 | 0.74 |
| C4N | 5 | -5.15 | 0.02 | 3.67 | 0.21 | 11.82 | 0.22 |
| C5N | 5 | -4.45 | 0.21 | 2.46 | 0.27 | 11.97 | 0.23 |
| C6N | 5 | -4.58 | 0.19 | 1.45 | 0.20 | 11.02 | 0.20 |
| P2' | 3 | 8.26 | 0.32 | 1.61 | 0.37 | 4.55 | 0.21 |
| OP1 | 3 | 8.14 | 0.53 | 1.73 | 0.94 | 3.60 | 0.75 |
| OP2 | 3 | 9.03 | 0.56 | 1.00 | 0.50 | 4.62 | 1.13 |
| OP3 | 3 | 8.62 | 0.79 | 2.41 | 1.40 | 4.94 | 0.68 |

Table 9D: Polypeptide and Solvent Interactors

| Acceptors | atom name | residue mol. # | residue # | total | x | ox | y | oy | z | oz |
|-----------|-----------|----------------|-----------|-------|------|-------|------|-------|-------|----|
| OE1 | GLU 1 | 181 | | -3.88 | | 5.25 | | | 14.75 | |
| OE1 | GLU 2 | 201 | | -4.15 | | 5.48 | | | 14.38 | |
| OE1 | GLU 3 | 163 | | -3.79 | | 3.89 | | | 15.77 | |
| OE1 | GLU 4 | 159 | | -3.14 | | 2.93 | | | 14.95 | |
| A11 | ACC | 11 | 4 | -3.74 | 0.43 | 4.39 | 1.20 | 14.96 | 0.59 | |
| OE2 | GLU 1 | 181 | | -4.37 | | 6.90 | | | 13.45 | |
| OE2 | GLU 2 | 201 | | -4.56 | | 6.92 | | | 12.74 | |
| A12 | ACC | 12 | 2 | -4.46 | 0.14 | 6.91 | 0.01 | 13.10 | 0.51 | |
| O | GLU 1 | 309 | | -8.06 | | 0.25 | | | 7.52 | |
| O | LEU 2 | 337 | | -7.71 | | -0.11 | | | 6.85 | |
| O | ALA 3 | 297 | | -7.26 | | -0.97 | | | 6.55 | |
| A21 | ACC | 21 | 3 | -7.67 | 0.40 | -0.28 | 0.63 | 6.97 | 0.49 | |
| OE2 | GLU 1 | 309 | | -4.36 | | -3.87 | | | 5.45 | |
| A23 | ACC | 23 | 1 | -4.36 | | -3.87 | | | 5.45 | |
| O | VAL 1 | 342 | | -7.20 | | 8.83 | | | 10.41 | |
| O | VAL 2 | 370 | | -6.94 | | 8.48 | | | 9.46 | |
| O | GLY 3 | 328 | | -6.79 | | 9.23 | | | 10.09 | |
| OE2 | GLU 4 | 183 | | -5.19 | | 8.47 | | | 10.50 | |
| O | ALA 5 | 365 | | -6.46 | | 8.51 | | | 10.35 | |
| A27 | ACC | 27 | 5 | -6.51 | 0.79 | 8.70 | 0.33 | 10.16 | 0.42 | |
| OD1 | ASP 3 | 179 | | 9.32 | | 1.02 | | | 6.96 | |
| A37 | ACC | 37 | 1 | 9.32 | | 1.02 | | | 6.96 | |
| OD2 | ASP 3 | 179 | | 8.04 | | 2.39 | | | 7.96 | |
| A38 | ACC | 38 | 1 | 8.04 | | 2.39 | | | 7.96 | |
| OH | TYR 3 | 188 | | -1.72 | | 2.70 | | | 6.02 | |
| A43 | ACC | 43 | 1 | -1.72 | | 2.70 | | | 6.02 | |

| Donors | atom name | residue mol. # | residue # | total | x | σx | y | σy | z | σz |
|--------|-----------|----------------|-----------|-------|-------|-------|-------|------|---|----|
| N | TYR 1 | 177 | | 0.42 | 4.12 | 9.29 | | | | |
| N | TYR 2 | 197 | | 0.54 | 3.95 | 9.16 | | | | |
| N | TYR 3 | 159 | | 0.39 | 3.86 | 9.94 | | | | |
| N | ASN 4 | 155 | | 0.81 | 4.22 | 10.27 | | | | |
| N | TYR 5 | 198 | | 0.50 | 4.45 | 10.69 | | | | |
| D8 | DON 8 | 5 | 0.53 | 0.17 | 4.12 | 0.23 | 9.87 | 0.65 | | |
| N | ILE 1 | 178 | | -0.30 | 3.00 | 11.99 | | | | |
| N | ILE 2 | 198 | | -0.19 | 3.01 | 11.87 | | | | |
| N | ILE 3 | 160 | | -0.46 | 2.46 | 12.45 | | | | |
| N | THR 4 | 156 | | -0.21 | 2.41 | 12.37 | | | | |
| D10 | DON 10 | 4 | -0.29 | 0.12 | 2.72 | 0.33 | 12.17 | 0.28 | | |
| NE | ARG 1 | 198 | | 10.97 | -1.63 | 5.67 | | | | |
| NE | ARG 2 | 218 | | 11.27 | -1.15 | 5.31 | | | | |
| NE | ARG 4 | 176 | | 11.22 | -1.28 | 5.21 | | | | |
| NE | ARG 5 | 222 | | 11.04 | -1.09 | 6.07 | | | | |
| D13 | DON 13 | 4 | 11.13 | 0.14 | -1.28 | 0.24 | 5.56 | 0.39 | | |
| NH1 | ARG 1 | 198 | | 11.24 | -3.80 | 4.93 | | | | |
| NH1 | ARG 2 | 218 | | 10.89 | -3.37 | 4.77 | | | | |
| NH1 | ARG 4 | 176 | | 10.67 | -3.32 | 4.21 | | | | |
| NH1 | ARG 5 | 222 | | 11.05 | -3.27 | 5.30 | | | | |
| D14 | DON 14 | 4 | 10.96 | 0.24 | -3.44 | 0.24 | 4.80 | 0.45 | | |
| NH2 | ARG 1 | 198 | | 9.54 | -2.45 | 4.11 | | | | |
| VAL 1 | ARG 2 | 218 | | 9.46 | -1.77 | 4.00 | | | | |
| NH2 | ARG 4 | 176 | | 9.50 | -1.43 | 3.70 | | | | |
| NH2 | ARG 5 | 222 | | 9.55 | -1.74 | 4.46 | | | | |
| D15 | DON 15 | 4 | 9.51 | 0.04 | -1.85 | 0.43 | 4.07 | 0.31 | | |
| NE | ARG 4 | 177 | | 10.99 | 4.32 | 2.39 | | | | |
| NH1 | ARG 1 | 204 | | 8.17 | 3.03 | 1.71 | | | | |
| NH1 | ARG 5 | 228 | | 7.75 | 1.68 | 1.45 | | | | |
| D18 | DON 18 | 3 | 8.97 | 1.77 | 3.01 | 1.32 | 1.85 | 0.48 | | |

| | | | | | |
|-----|-------|-----|------|-------|-------|
| N | GLY 1 | 262 | 2.72 | -3.76 | 9.55 |
| N | GLY 2 | 290 | 2.62 | -3.74 | 9.51 |
| N | GLY 3 | 243 | 2.38 | -4.07 | 9.32 |
| N | GLY 4 | 244 | 1.45 | -3.80 | 10.09 |
| N | GLY 5 | 286 | 2.74 | -3.85 | 9.80 |
| D23 | DON | 23 | 5 | 2.38 | 0.13 |
| OH | TYR 3 | 188 | | -1.72 | 2.70 |
| D46 | DON | 46 | 1 | -1.72 | 2.70 |
| NH1 | ARG 4 | 181 | | 3.70 | 2.30 |
| D58 | DON | 58 | 1 | 3.70 | 2.30 |
| ND2 | ASN 4 | 260 | | -5.70 | 2.24 |
| D62 | DON | 62 | 1 | -5.70 | 2.24 |

Waters

| ters | name | residue. | residue # | total | x | σx | y | σy | z | σz |
|------|-------|----------|-----------|-------|-------|------|-------|------|-------|----|
| | | mol. | # | | | | | | | |
| O | HOH 1 | 35 | | | 0.68 | | -3.50 | | 12.51 | |
| O | HOH 2 | 511 | | | 0.54 | | -3.42 | | 12.61 | |
| O | HOH 3 | 461 | | | -0.15 | | -4.12 | | 12.26 | |
| WAT | 2 | 3 | 0.36 | 0.44 | -3.68 | 0.38 | 12.46 | 0.18 | | |
| O | HOH 1 | 70 | | | 2.74 | | 1.12 | | 10.80 | |
| O | HOH 2 | 524 | | | 3.09 | | 1.48 | | 10.72 | |
| O | HOH 3 | 901 | | | 2.86 | | 1.06 | | 11.09 | |
| O | HOH 4 | 618 | | | 3.03 | | 0.85 | | 11.05 | |
| WAT | 4 | 4 | 2.93 | 0.16 | 1.13 | 0.26 | 10.91 | 0.18 | | |
| W4 | | 115 | | | -9.62 | | 7.01 | | 9.04 | |
| O | HOH 1 | 514 | | | -9.26 | | 6.65 | | 7.93 | |
| O | HOH 2 | 499 | | | -8.71 | | 7.08 | | 8.17 | |
| O | HOH 3 | 861 | | | -9.99 | | 6.36 | | 10.10 | |
| O | HOH 4 | 121 | | | -9.33 | | 7.20 | | 8.93 | |
| O | HOH 5 | 6 | 5 | -9.38 | 0.47 | 6.86 | 0.35 | 8.83 | 0.85 | |
| WAT | | | | | | | | | | |
| W6 | HOH 1 | 171 | | | 0.30 | | 3.54 | | 6.18 | |
| O | HOH 2 | 984 | | | 0.61 | | 3.27 | | 5.33 | |

Table 10A: Pharmacofamily 8 Subset

| Molecule # | pdb | type | rmsd from family avg. |
|------------|-------------------|----------------------------|--------------------------------|
| 1 | 1QGA | Ferrodoxin Reductase (pea) | 0.61 |
| 2 | P450 ¹ | P450 reductase (rat) | 0.35 |

Table 10B: Polypeptide and Solvent Interactors (average coordinates)

| Acceptors | | | total | x | σx | y | σy | z | σz |
|-----------|--------------------|--|-------|--------|------|-------|------|--------|------|
| atom name | residue. mol. # | | | | | | | | |
| A2 | ACC | | 2 | 0.63 | 0.38 | -6.60 | 0.21 | -7.09 | 0.16 |
| A8 | ACC | | 2 | -2.87 | 0.25 | -3.55 | 0.64 | -0.51 | 0.02 |
| A11 | ACC | | 2 | -4.28 | 0.30 | 8.10 | 0.34 | 3.52 | 0.33 |
| A14 | ACC | | 2 | -7.58 | 0.10 | 8.62 | 0.24 | 3.69 | 0.19 |
| A18 | ACC | | 2 | -12.53 | 0.11 | 8.89 | 0.59 | 0.72 | 0.62 |
| A21 | ACC | | 2 | -8.28 | 0.08 | 9.45 | 0.25 | -6.25 | 0.84 |
| A23 | ACC | | 2 | -1.15 | 0.00 | -2.54 | 0.21 | -7.56 | 0.09 |
| A29 | ACC | | 2 | -1.63 | 0.84 | -6.66 | 0.42 | -10.70 | 0.06 |
| A31 | ACC | | 2 | -7.49 | 0.70 | -5.59 | 0.66 | -9.88 | 0.66 |
| A32 | ACC | | 1 | -8.95 | - | -3.74 | - | -4.78 | - |
| Donors | | | total | x | σx | y | σy | z | σz |
| atom name | residue. mol. # | | | | | | | | |
| D2 | DON | | 2 | 0.63 | 0.38 | -6.60 | 0.21 | -7.09 | 0.16 |
| D4 | DON | | 2 | -6.69 | 0.23 | -1.87 | 0.78 | 5.73 | 0.27 |
| D8 | DON | | 2 | -1.98 | 0.25 | -0.80 | 0.53 | -0.07 | 0.05 |
| D9 | DON | | 2 | -2.87 | 0.25 | -3.55 | 0.64 | -0.51 | 0.02 |
| D15 | DON | | 2 | -7.58 | 0.10 | 8.62 | 0.24 | 3.69 | 0.19 |

| | | | | | | | | |
|-----|-----|---|--------|------|------|------|-------|------|
| D18 | DON | 2 | -10.73 | 0.10 | 5.15 | 0.70 | 6.85 | 0.21 |
| D21 | DON | 2 | -12.39 | 0.55 | 8.95 | 0.83 | 4.42 | 0.46 |
| D23 | DON | 2 | -12.53 | 0.11 | 8.89 | 0.59 | 0.72 | 0.62 |
| D26 | DON | 2 | -10.08 | 0.70 | 9.97 | 0.39 | -5.61 | 0.35 |

Table 10C: NAD(P) Conformer Model

| atom name | number | x | σx | y | σy | z | σz |
|-----------|--------|--------|------|-------|------|-------|------|
| PA | 2 | -6.90 | 0.19 | 1.29 | 0.01 | 2.19 | 0.44 |
| O1A | 2 | -8.23 | 0.13 | 0.84 | 0.28 | 2.29 | 1.01 |
| O2A | 2 | -6.22 | 0.68 | 1.25 | 0.00 | 3.45 | 0.19 |
| O5'A | 2 | -6.94 | 0.05 | 2.74 | 0.01 | 1.67 | 0.46 |
| C5'A | 2 | -5.96 | 0.32 | 3.31 | 0.21 | 0.99 | 0.16 |
| C4'A | 2 | -6.21 | 0.28 | 4.77 | 0.19 | 0.81 | 0.08 |
| O4'A | 2 | -7.07 | 0.21 | 4.93 | 0.07 | -0.33 | 0.12 |
| C3'A | 2 | -6.95 | 0.32 | 5.45 | 0.19 | 1.99 | 0.09 |
| O3'A | 2 | -6.38 | 0.22 | 6.74 | 0.20 | 2.25 | 0.09 |
| C2'A | 2 | -8.36 | 0.28 | 5.60 | 0.08 | 1.51 | 0.12 |
| O2'A | 2 | -9.02 | 0.09 | 6.71 | 0.01 | 2.15 | 0.10 |
| C1'A | 2 | -8.10 | 0.23 | 5.82 | 0.11 | 0.05 | 0.07 |
| N9A | 2 | -9.26 | 0.18 | 5.67 | 0.07 | -0.81 | 0.09 |
| C8A | 2 | -10.48 | 0.15 | 5.08 | 0.02 | -0.58 | 0.05 |
| N7A | 2 | -11.35 | 0.01 | 5.15 | 0.09 | -1.61 | 0.14 |
| C5A | 2 | -10.62 | 0.05 | 5.84 | 0.01 | -2.55 | 0.11 |
| C6A | 2 | -10.98 | 0.07 | 6.27 | 0.00 | -3.84 | 0.10 |
| N6A | 2 | -12.17 | 0.06 | 6.02 | 0.00 | -4.36 | 0.08 |
| N1A | 2 | -10.08 | 0.13 | 6.95 | 0.04 | -4.59 | 0.09 |
| C2A | 2 | -8.88 | 0.12 | 7.22 | 0.07 | -4.10 | 0.04 |
| N3A | 2 | -8.46 | 0.02 | 6.87 | 0.15 | -2.90 | 0.02 |
| C4A | 2 | -9.35 | 0.07 | 6.17 | 0.04 | -2.06 | 0.07 |
| O3 | 2 | -6.11 | 0.32 | 0.30 | 0.20 | 1.21 | 0.13 |
| PN | 2 | -5.73 | 0.14 | -1.29 | 0.24 | 1.48 | 0.01 |
| O1N | 2 | -6.50 | 0.06 | -1.63 | 0.42 | 2.69 | 0.13 |
| O2N | 2 | -4.30 | 0.14 | -1.48 | 0.06 | 1.62 | 0.06 |

| | | | | | |
|------|--------|------|-------|------|-------|
| O5'N | -6.26 | 0.37 | -2.13 | 0.26 | 0.26 |
| C5'N | -5.67 | 0.29 | -2.09 | 0.15 | -1.01 |
| C4'N | -6.63 | 0.26 | -2.81 | 0.33 | -1.93 |
| O4'N | -6.11 | 0.28 | -2.90 | 0.27 | -3.27 |
| C3'N | -6.95 | 0.06 | -4.24 | 0.38 | -1.45 |
| O3'N | -8.35 | 0.03 | -4.47 | 0.60 | -1.50 |
| C2'N | -6.22 | 0.01 | -5.16 | 0.30 | -2.41 |
| O2'N | -7.01 | 0.15 | -6.29 | 0.42 | -2.74 |
| C1'N | -5.90 | 0.11 | -4.29 | 0.22 | -3.62 |
| MN1 | -4.55 | 0.05 | -4.52 | 0.01 | -4.21 |
| C2N | -4.50 | 0.03 | -5.07 | 0.06 | -5.47 |
| C3N | -3.29 | 0.08 | -5.32 | 0.10 | -6.13 |
| C7N | -3.24 | 0.24 | -5.90 | 0.02 | -7.52 |
| O7N | -3.24 | 1.75 | -6.01 | 0.02 | -8.11 |
| MN7 | -3.18 | 1.32 | -6.31 | 0.10 | -8.11 |
| C4N | -2.09 | 0.01 | -5.00 | 0.39 | -5.44 |
| C5N | -2.15 | 0.06 | -4.44 | 0.46 | -4.14 |
| C6N | -3.40 | 0.11 | -4.21 | 0.25 | -3.54 |
| P2' | -10.21 | 0.02 | 6.47 | 0.10 | 3.22 |
| OP1 | -10.72 | 1.21 | 5.88 | 0.71 | 3.20 |
| OP2 | -10.31 | 0.01 | 7.62 | 0.12 | 4.24 |
| OP3 | -10.73 | 1.02 | 5.69 | 1.01 | 3.24 |

Table 10D: Polycaprolide and solvent Interactors

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| Donors | atom name | residue mol. # | residue # | total | x | ox | y | oy | z | sz |
|--------|-----------|----------------|-----------|--------|------|-------|------|-------|------|----|
| OG | SER 1 | 90 | | 0.366 | | -6.74 | | -6.97 | | |
| OG | SER 2 | 457 | | 0.899 | | -6.45 | | -7.20 | | |
| D2 | DON | 2 | 2 | 0.633 | 0.38 | -6.60 | 0.21 | -7.09 | 0.16 | |
| NZ | LYS 1 | 110 | | -6.847 | | -2.42 | | 5.92 | | |
| NH1 | ARG 2 | 298 | | -6.526 | | -1.32 | | 5.54 | | |

Throughout this application various publications have been referenced. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to 5 more fully describe the state of the art to which this invention pertains.

Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific details 10 are only illustrative of the invention. It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Therefore, it should be understood that 15 various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.